ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

THE KEEPING QUALITY OF COW'S MILK AND MOTHER'S MILK

INVESTIGATIONS WITH REFERENCE TO TEMPERATURE, HEAT TREATMENT, CHANGES IN FLAVOUR AND pH, AND THE LYSOZYME CONTENT

BY

ESTER SELESTE

VOL. 31 1953 SUPPLEMENTUM 8

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM HELSINKI UNIVERSITY CHILDREN'S CLINIC, CHIEF: PROF. ARVO YLPPÖ, M. D., ARCHIATER, AND DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, CHIEF: PROF. K. O. RENKONEN, M. D.

THE KEEPING QUALITY OF COW'S MILK AND MOTHER'S MILK

INVESTIGATIONS WITH REFERENCE TO TEMPERATURE, HEAT TREATMENT, CHANGES IN FLAVOUR AND pH, AND THE LYSOZYME CONTENT

BY

ESTER SELESTE

TO BE PUBLICLY DISCUSSED, BY PERMISSION OF THE MEDICAL FACULTY OF THE UNIVERSITY OF HELSINKI, IN AUDITORIUM XII ON SEPTEMBER 23 1953, AT 12 O'CLOCK NOON

HELSINKI 1953

Turku . 1953
Uuden Auran Osakeyhtiön kirjapaino

PREFACE

It is my pleasant duty in this connection to thank my respected teacher, the Chief of Helsinki University Children's Clinic, Prof. Arvo Ylppö, M. D., Archiater, who suggested the subject of this thesis and has shown an interest in and proffered advice on the work.

My warmest thanks are due to the Chief of the Helsinki University Department for Serology and Bacteriology, Prof. K. O. Renkonen, M. D., for the unstinting help and instruction he has given me throughout the period of this investigation.

To Prof. Torsten Storgårds, Professor of Dairy Science at Helsinki University, I extend my thanks for the valuable practical directives he has given me.

I am greatly indebted to Mr. Martti Helske, student of chemistry, Mr. Antti Louhivuori, M. Sc., and Mr. Olof Forsander, M. Sc., for the invaluable help they have given me in the technical and mathematical treatment of the material, and to Dr. Ilari Rantasalo, M. D., for his kind guidance in working out the bacteriological nomenclature.

Also, I wish to express my deep gratitude to all those who have helped me in connection with my laboratory work, particularly Mrs. Ann-Mari Karsten, laboratory assistant, and nurse Irja Martikainen. I am very grateful, too, to all those who have been helpful in procuring the milk samples, especially Miss Aino Myyryläinen, Public Health Nurse, and Miss Gerda Olenius of the Rosenlund Farm.

My best thanks are due to Mrs. Helvi Lehmus for her generous assistance in retyping the manuscript.

Finally, I am indebted for the translation of my work to Mrs. Hilkka Kontiopää, M. A. (Helsinki) and to Mr. L. A. Keyworth, M. A. (Cantab.).

Helsinki, June 1953.

Ester Seleste

CONTENTS

I. INTRODUCTION

So-called well baby clinics have been introduced recently in most countries. As they are often combined with mother's milk centres collecting considerable quantities of mother's milk from quite large areas, storing and distributing it to needful diseased infants, the study of problems connected with the storage and keeping qualities of mother's milk has become particularly topical. The purpose of the present investigation is to shed additional light on the keeping quality of primarily breast milk, but also of cow's milk, taking into account storage temperatures and methods of treatment, and changes in the flavour and acidity (pH) of the milk.

Mother's milk centres are necessary and very beneficial in urban areas where many mothers, for one reason or another, cannot themselves feed their babies and other mothers have breast milk surplus to the requirements of their own babies.

The general rule is that the milk of a particular animal species is the best possible for the offspring of that species. The truth of this assertion is not disproved by the fact that in certain countries, e. g. the United States, where the general hygiene level is high, cow's milk preparations can be successfully used for feeding infants (MACKINTOSH [137]). It is a well known fact that human milk can be better utilized than cow's milk (MELLANDER [141]).

The lives of seriously diseased infants especially and premature babies in particular can now often be saved in Finland also thanks alone to the milk supplies held by mother's milk centres (YLPPÖ [215]).

It is evident that in conditions where cow's milk is not first-class its use may cause a great deal of harm. The same can be said, though much less often, of human milk in cases where the hygiene at home is not good and where the mothers have not shown sufficient care in the taking and handling of breast milk.

In 1911 in Boston 62 infants out of 100 were fed with breast milk and 38 with bottle milk; of 621 infants who died of diarrhoea during this time 87 had been breast-fed and 534 bottle-fed. The reason established was the heightened risk of infection to which the cow's milk was exposed in connection with all the different stages of handling in large-scale milk production. Mother's milk, in either direct or indirect feeding, is exposed to less risk of contamination (Garland [75], Scheuer et al.[175], Gairdner [71]).

It has been found that milk-borne diseases derive partly from a diseased animal and partly from a diseased human being (Savage [173], Adametz [1], Strauss [188], Kelley et al. [120], Garland [72, 73, 74, 75], Brooke [25], Huebner et al. [102, 103], Jellison et al. [106], Beck et al. [15], Kästli [130]), but infections may also appear in and spread by the route man-cow-man, as was found e. g. in connection with the septic angina epidemic in Portland in 1922 (Garland [74]).

As mother's milk is distributed in small quantities severe epidemics, like cow's milk epidemics, are hardly likely. In children's hospitals using large quantities of milk stored by breast milk centres, such epidemics may of course occur. No reports of such epidemics have been found in the literature but where mother's milk is unhygienic it naturally causes damage in casu.

It can perhaps be said today that if cow's and mother's milk is absolutely hygienically collected and stored the question of epidemics need not arise.

The bacteriological aspects of this problem have been the subject of intense investigation mainly because of infections borne by cow's milk. As no parallel investigations into the pH and flavour of milk, as indicators of its keeping quality, have yet been effected an attempt will be made in the present investigation to illustrate this side of the matter too.

II. THE PROBLEM

As mentioned at the outset the starting-point was to observe the keeping quality and methods of storage of the breast milk received by mother's milk centres.

To start with observations were made of how breast milk keeps in the natural state, and how various treatments effect this keeping quality. For the sake of comparison a parallel study has been made of the effect of the same factors on cow's milk. Attention has been devoted to the following points:

1. How raw milk, Holder-Time pasteurized milk (62°C — 20 min.), and HTST (High-Temperature Short-Time) pasteurized (80°C — 2 min.) milk kept at different temperatures react as regards changes in pH and flavour, and what method of treatment is the most favourable.
2. Whether conclusions can be drawn from pH variations as to the quality and keeping quality of the milk concerned.
3. Whether the quality of the milk can be judged from its flavour.
4. Whether there is any correlation between the flavour and the pH values.
5. The importance of Streptococcus lactis in the pH variations of milk.
6. The importance of Escherichia coli in the pH variations of milk.
7. Whether lysozyme has any effect on the keeping quality of milk.

Before embarking on a discussion of the above questions the author gives a detailed survey of the sum of knowledge of the hygiene, smell, flavour, storage and heat treatment of cow's and human milk.

III. REVIEW OF LITERATURE I

HYGIENE OF MILK

A. Bacteriology of cow's milk

A comparison of human and cow's milk, primarily their environmental conditions, from the hygienic point of view shows that the natural bacterial content of cow's milk is, understandably, much higher. Apart from the ordinary air bacteria, cow's milk is also exposed to all kinds of hay bacteria and the bacteria, often apathogenic to man, deriving from the cow's skin and manure (GOTSCHLICH [79]). Naturally microbes pathogenic to man can also enter into question, from a diseased cow. Milking adds another source of infection: man (ROWLANDS [168], etc.).

The risk of infection is increased by the process of milking unless cleanliness is closely observed. Unskilled and dirty milking techniques introduce enormous numbers of bacteria into the milk. Of equal importance is the hygiene of the equipment. Unless the pails are properly dried after washing bacteria rapidly grow in profusion on any surface where a thin moist film invisible to the eye is left, and infect the milk (BARTHEL [10]).

Most of the so-called milk-borne epidemics derive from such insanitary handling of milk or from a bacillus carrier. No wonder, then, that systematical dairy inspection was introduced as early as 1906 — in the U.S.A. — and public health authorities began to discuss the milk problem in its various aspects, e.g. as a source of disease (GARLAND [72]).

The author has picked out the following milk-borne epidemics from the literature:

In 1857, (according to SAVAGE [173]) typhoid fever epidemics spread by milk were recorded in England. In 1912 Chicago had

10,000 cases of epidemic streptococcus angina due to raw milk. ADAMETZ [1] found that both epidemics and epizootics were often attributable to milk. The root causes were: unclean milking, milk receptacles, cowshed air, adulteration, and diseased cow. In 1892 STRAUSS [188] began to distribute pasteurized milk in New York City; infant mortality in the summer was then 136.4 pro mille but 15 years later it had dropped to 62.7 pro mille. In 1922 Portland, Oregon had 487 cases of septic angina, 22 of them fatal; the epidemic was found to have affected almost exclusively people who received their milk from one particular raw milk dairy (GARLAND [74]). According to KELLEY et al. [120] milk is practically never the source of diphtheria; milk-borne scarlet fever is more common, but commonest of all milk-borne diseases is typhoid fever. Septic sore throat is evidently almost always due to infected milk. As regards infant mortality and diarrhoea, GARLAND [74] says that "there can be no question but that milk is intimately connected with infant mortality, entering prominently into the following of its causes: diarrheal diseases, acute contagion and tuberculosis".

Milk-borne epidemics have occurred also in Glasgow (BROOKE [25]), such as the typhoid fever and diphtheria epidemics in 1936, paratyphoid fever epidemic in 1937, and Flexner's dysentery in 1940. It was found that the milk was contaminated by men, in the first place the milkers. After 1947 the Q-fever epidemics in South California were studied (BECK at al. [15], HUEBNER et al. [102, 103], JELLISON et al. [106]) and it was found that the source of infection was the milk of certain dairies. In Finland also the incidence of diarrhoea has been found to be higher with bottle-fed babies than breast-fed babies (YLPPÖ et al. [216], HALLMAN [85]). The only milk-borne epidemics known from Finland are the scarlet fever of 1929 and 1939, the paratyphoid fever of 1945 (RANTASALO [161 a]), and the diphtheria of 1948 (RANTASALO [161 b]).

Infection of milk may cause a simple decaying process only or may introduce into the milk many agents of disease pathogenic to man. It is perhaps impossible always to define clearly the borderline between the two types.

The bacteria present in milk are divided up into the following categories: (a) the normal bacterial flora of the milk regularly present in ordinary commercial milk, (b) the saprophytes occa-

sionally entering the milk and causing abnormal changes, and (c) pathogens which gain access to the milk either from diseased milch animals or later by contact, directly or indirectly, from diseased or latently infected men, polluted water, unclean equipment etc. (KOLLE [126], GOTSCHLICH [79]).

The normal bacterial flora of the milk includes the following bacteria (GOTSCHLICH [79]):

1. Aerobic lactic acid bacteria, a group including Streptococcus acidi lactici, Aerobacter aerogenes, and the lactic acid bacteria producing coagulation (''Säure-Labbildner''). (Wherever feasible the bacteria are identified according to BERGEY's Manual of Determinative Bacteriology [22]).

2. Anaerobic lactic acid bacilli which decompose milk very distinctively, developing a very bad smell. Hence no one considers such milk edible.

3. Aerobic peptonizing bacteria which decompose milk almost imperceptibly, in the early stages in particular; hence such milk retains throughout a normal appearance and is consumed without suspicion (FLÜGGE [67], GOTSCHLICH [79]). However, such apparently completely unchanged milk may produce severe toxic effects.

The saprophytes occasionally getting into the milk are mainly responsible for the so-called ''milk defects''. Among them may be mentioned the slime-formers and the colour-formers (GRIMMER [81]). This group includes further the fat-decomposing bacteria which produce the soapy and fishy flavours encountered in milk, and the peptonizing bacteria responsible for its bitter taste (GRIMMER [81]).

Among the pathogenic microbes entering milk may be mentioned (SAVAGE [173], ADAMETZ [1], STRAUSS [188], KELLEY et al. [120], GARLAND [72, 73, 74, 75], BROOKE [25], HUEBNER et al. [102, 103], JELLISON et al. [106], BECK et al. [15]):

(a) Among the milk infections deriving from a diseased animal are scarlet fever, bovine tuberculosis, viz. tuberculosis of the udder, brucellosis, smallpox, Q-fever, septic angina, foot and mouth disease, anthrax and various diarrhoeal conditions induced in children by E. coli and other bacteria.

(b) Man can infect the cow with certain pathogenic bacterial strains causing diseases such as scarlet fever, septic angina and other streptococcus and micrococcus infections, human

tuberculosis and diphtheria, not to mention the Salmonella infections which like the other above-mentioned infections can further spread via cow's milk to man.

A factor which is of great importance in connection with the bacterial flora of the milk is the storage temperature. PARK [154] found that the bacterial count of cow's milk declined with storage at 42°F (5.5°C), increased moderately at 50°F (10°C) and considerably when the storage temperature reached room or incubator temperature. The figures were: original number of bacteria = 5,000/cc, after 24 hours at 42°F (5.5°C) = ad 2,400, at 50°F (10°C) = ad 7,000, at 65°F (18.3°C) = ad 208,000, and at 95°F (35°C) = ad 12,500,000,000.

BROCK [24] reports the following investigation result:

Bacterial count in 1 cc of cow's milk

	15°C	25°C	35°C
At the beginning of the investig. ..	9,300	9,300	9,300
After 3 hours	10,000	18,000	30,000
„ 6 „	25,000	172,000	12,000,000
„ 9 „	46,000	1,000,000	35,000,000
„ 24 „	5,700,000	50,000,000	577,500,000

Different temperatures favour the development of different bacterial groups. Mainly fluorescent bacteria develop at temperatures under + 5°C, Proteus vulgaris, micrococci and alkali-forming short rods at + 5—10°C, at + 10—15°C Streptococcus lactis begins to proliferate greatly and at + 15—30°C this bacterium dominates completely. At + 30—40°C also E. coli and A. aerogenes, lactobacilli and the yeast fungi decomposing lactose develop (BARTHEL [10], KOLLE [126], GUBITZ [82] etc.).

Hence the quality of milk is greatly dependent on bacteria and their number is influenced by the storage temperature, a fact that has long been devoted special attention by the dairy industry.

B. Bacteriology of mother's milk

The primary bacterial flora of human milk is much scantier than that of cow's milk. However, sucked human milk is not completely sterile for the bacteria present on the body surface are

naturally present at the mouth of the ducts of the mammary gland and at the nipple. As long as they include no pathogens these bacteria with access to the milk are of no practical importance but a diseased mother can of course pass on pathogenic bacteria to a baby in suckling it.

According to FEER [59] the infant may suck even a mastitis breast as long as no suppuration occurs, ''da das Schlucken von Frauenmilch, der infektiöser Eiter beigemengt ist, unschädlich ist''. However, cases have been found, e.g. at Helsinki University Children's Clinic (not published), of the infant contracting recurrent diarrhoea every time it has had from its mother (with a completely healthy breast) milk which contained Micrococcus pyogenes var. aureus.

COHN et al. [38] found that breast milk usually contains pus cocci, particularly M. pyogenes var. albus, of external origin and most numerous in the peripheral parts of the glandular ducts. HONIGMANN [99] found in mother's milk mostly M. pyogenes var. albus but also, though slightly less in number, M. pyogenes var. aureus; other bacterial species, if found at all, were seldom present and very few in number. In his opinion, which is shared by HESSE [92] and BASENAU [12], the bacteria present in breast milk probably get into the milk ducts from the skin through the duct apertures of the nipples. GRIMMER [81] found in breast milk practically only M. pyogenes var. albus, seldom M. pyogenes var. aureus. In the milk of mothers in confinement ESCHERICH [55] found micrococci only. In cases where the breasts were intact he believed that the bacteria had entered the breast via the bloodstream.

DUNCAN et al. [52] found that M. pyogenes var. aureus is common in the milk of healthy lactating mothers and in the throat and intestines of their infants; in one hospital 82 out of 87 mothers and infants, 90 per cent, were affected. The virulence of the cocci seemed to be low for in spite of swallowing large quantities of bacteria with the milk the children did not become diseased. In the majority of cases the cocci seemed first to appear in the throat of the infant, with the infants' ward as the possible source of infection, and to spread from infant to infant or infant to mother and back through mother's milk. With the 87 mothers and infants examined M. pyogenes var. aureus was first detected in the infant's

throat in 33 cases, first in the breast milk in 15 and simultaneously in infant and mother in 39 cases. The same M. pyogenes var. aureus strain was found to grow in breast milk and in the throat and rectum.

The bacteriological study of breast milk has been occasional and disconnected in character on the whole. Since the establishment of the first breast milk centre in Boston in 1910 (MACPHERSON et al. [138], WRIGHT [204, 205]) these centres have gradually increased in number; at the same time increasing attention has been devoted to the bacteriology and secondary infection of breast milk.

Among the common bacteria present in milk E. coli is the most important pathogenic agent in the secondary infection of breast milk, particularly in infantile diarrhoea (FEER [59] and WRIGHT [204], etc.). The presence of this bacterium in breast milk is an indication of the unhygienic treatment of the milk, as pointed out before, and such milk is not accepted for distribution by mother's milk centres (CHRISTENSEN [35], WRIGHT [204], FEER [59] etc.).

SAUER [172] cultivated E. coli and saprophytic bacteria from breast milk, even after pasteurization. POETSCHKE [158] demonstrated E. coli in 68.7 per cent and M. pyogenes var. aureus in 50 per cent of his cases; in 20 samples of breast milk from two donors A. aerogenes grew once and Pseudomonas aeruginosa once. WRIGHT [204, 205] often found in breast milk micrococci capable of forming toxins if the milk was kept in unfavourable conditions, e.g. at room temperature. Micrococci were the most frequent bacteria found followed by haemolytic and non-haemolytic streptococci. M. pyogenes var. aureus was found in 18 out of 95 cases. Milks kept in unsterilized receptacles often contained coliform bacteria (E. coli, A. aerogenes and Alcaligenes faecalis). CHRISTENSEN [35] found colibacilli in the breast milk of 18 per cent of the donors he examined, haemolytic streptococci never. In mastitis cases the commonest bacterium found by DUDGEON et al. [50] was M. pyogenes var. aureus, followed in the order of incidence by M. pyogenes var. albus, streptococci and E. coli. The commonest species that normal breast milk also may contain was, they found, M. pyogenes var. albus, with M. pyogenes var. aureus and E. coli, either one or both of them, next. DUNCAN et al. [52] came to the same conclusion concerning M. pyogenes var. aureus.

STORAGE AND HEAT TREATMENT OF MILK

A. Cow's milk

From the milk hygiene point of view the abdominal and intestinal diseases of the milch animals are of importance. It is a known fact that the milk of cows affected with acute or chronic diarrhoea often causes vomiting and diarrhoea in infants. This has been attributed to the more abundant growth in this milk of E. coli and its toxins. It must be borne in mind that the effect of the toxin is observable even after the milk has been heated (GARLAND [72]).

As milk has been found to spread infection and as the pathogens can also multiply in milk its treatment and storage constitute a hygienic problem of the greatest importance. Aseptic handling, the necessary and rapid cooling immediately after milking, sufficiently low storage temperature and the killing of pathogens (pasteurization) are very important factors (PARK [154], GUBITZ [82], JIMISON [107], JOHANNSEN [108], FLÜGGE [67], ESCHERICH [56], FEER [59] and MASON [139]).

Milk, it is true, has its special bacteriostatic and bactericidal principle, classed among the lysozyme-type principles. However, as its bactericidity is fairly weak (JONES [111]) the above-mentioned asepsis, cooling and heat treatment are of very great importance.

Pasteurization is a measure for the heating of thermolabile products only to the degree necessary to ensure that certain types of micro-organisms present in them either die or are inhibited from too viable proliferation, however without aspiring to complete sterility or spoiling the product treated (McCULLOCH [140]). PASTEUR [155] in 1860 was the first to use moderate heating to find out the extent to which unknown yeasts and bacteria were responsible for the souring of wine.

Soon after PASTEUR's successful experiments an act was passed in Denmark stipulating that all whey and skimmed milk was to be heated before it was returned to farms to be fed to calves and pigs. The purpose was to prevent the spread of tuberculosis and other diseases from infected cows. Pasteurization orginally aimed at protecting animals, not men, against infections (McCULLOCH [140]).

Now came the development of various pasteurization and steril-

ization methods in the storing of cow's milk. Results and opinions differed widely. Unanimity prevailed on one point — that milk had to be heated in some way (HESSE [92], FLÜGGE [67], BASENAU [11], TITUS [193], KITCHEN [123]). But still the use of raw milk was favoured by some (HESSE [93], CHAPIN [34]).

Inadequate automatic control of the time and temperature factors in the heating process and irresponsible operators who failed to appreciate the importance of sufficient heat treatment led in 1900 to the substitution of ''Holder-Time'' pasteurization for the ''flash'' pasteurization practised up to then. Many milk-borne epidemics of this time could be traced back to ''flash'' pasteurized milk. ''Flash'' pasteurization is the momentary heating of milk to a high not recorded temperature. It was replaced by HTST pasteurization (HTST = High-Temperature Short-Time), heating the milk at a certain temperature for a certain time.

The U. S. Public Health Service Milk Ordinance and Code [161] states: ''The terms 'pasteurization', 'pasteurized', and similar terms shall be taken to refer to the process of heating every particle of milk or milk products to a temperature of not less than 142°F, and holding at such temperature for not less than 30 minutes in approved pasteurization apparatus, provided that approval shall be limited to apparatus which requires a combined holder and indicating thermometer temperature tolerance of not more than 1 ½ °F, as shown by official tests with suitable testing equipment, and provided that such apparatus shall be properly operated and that the indicating thermometers and the recording thermometer charts both indicate a temperature of not less than 143 ½ °F, continuously throughout the holding period. The terms 'pasteurization' 'pasteurized' and similar terms shall also include the process of heating every particle of milk or milk products to 160°F, and holding at that temperature or above for not less than 15 seconds in apparatus of approved design and properly operated. Provided that nothing contained in this definition shall be construed as disbarring any other process which has been demonstrated as of at least equal efficiency and is approved by the State health authority.''

HTST pasteurization is much used in present-day dairy work — either the so-called low pasteurization method (70—71°C — 15

seconds) or the high pasteurization (80°C — 2 minutes) — but long-time pasteurization or Holder-Time pasteurization is also popular (60—62°C — 30 minutes). Opinions differ as to the superiority of these methods; some investigators prefer HTST pasteurization (ANDERSSON et al. [6], HOLMQVIST [97], GARLAND [72, 73, 74, 75], JIMISON [107], HUEBNER et al. [102, 103]); DOTTERER [47, 48] favours Holder pasteurization and SEIBEL et al. [180] are against it, whereas others have found no great difference between the two methods (MILLENKY et al. [145], FABIAN [58], WILSON [207], BROOKE [25], KÄSTLI [130]). Further information can be found in the detailed monograph by McCULLOCH [140].

Several investigations have been made into the effect of heat treatment on the bacteria in cow's milk (FLÜGGE [67], SEIBEL et al. [180], BREED [21], HUCKER [101], BUCHBINDER et al. [27, 28], FISCHER [60], DE SORIANO [186], HUEBNER et al. [102, 103], RITTER [163], HILEMANN et al.[94]).

B. Mother's milk

TALBOT [192], the founder of the first (1910) mother's milk centre, showed that mother's milk could be collected and stored at a mother's milk centre without detriment and distributed to infants. In 1939 MACPHERSON et al. [138] published their "Standard for Directories for mother's milk", in which they discussed the questions of organization, staff, milk donors, collection, plant and equipment, treatment, records and reports connected with mother's milk. In 1942 SMITH [183] published "Human milk's technology", reporting on the practical measures of treatment, identification, pasteurization, and storage of mother's milk and the effect of treatment on the nutritional value. The organization and operation of mother's milk centres have been reported on by WRIGHT [204, 205], CHAPIN [34], SALMI [171], v. SYDOW [190], LAWS et al. [132, 133, 134], etc.

Several investigations have been made into the hygiene of mother's milk. The conclusions drawn are the same as for cow's milk, viz. that aseptic handling, the necessary and rapid cooling immediately after expressing the milk and sufficiently low storage temperature are factors of great importance (AMERICAN ACADEMY OF PEDIATRICS [4], DYNSKI-KLEIN [53], CHRISTENSEN [35], WRIGHT [204, 205], v. SYDOW [190] and ESTEVES [57]).

Like cow's milk, mother's milk must also be cooled rapidly immediately after milking and kept in a sufficiently cool place, preferably in a refrigerator. According to MACKINTOSH [137] the milk must be cooled at once to 40°F (4.4°C) and only then added to the stored consignment. In reality, in Finland at least, very few homes have a refrigerator, and the temperature of pantries in the summer leaves a great deal to be desired. Also, not all mothers cool their milk; a large number, in spite of instructions to the contrary, express milk direct into existing stored milk. Hence the milk is brought up to room temperature several times a day, during which time the bacteria increase readily and rapidly in such good culture medium. The result is seen when the milk is tested at mother's milk centres where the bacterial count of the milk is taken (LEISTI [136]). Some mothers produce practically sterile milk while the milk of others may contain even several million bacteria to a millilitre. How much the lack of hygiene and insufficient cooling can detract from the purity of breast milk has become evident from several investigations (LEISTI [136], v. SYDOW [190], SCHERER [174], WRIGHT [204, 205], WILSON [207]).

As mentioned above, dairies have generally adopted HTST pasteurization. The methods of pasteurizing mother's milk still vary. Most countries and particularly Scandinavia favour the Holder-Time pasteurization method. In Denmark breast milk is pasteurized at 69°C for 10 minutes, in Sweden at 68°C for 10 minutes, in Finland e.g. at the Children's Clinic at 63°C for 20 min., in the Children's Castle at 70°C for 10 min., at the ''Maitopisara'' (Milk Drop) mother's milk centre at 80°C for 2—3 min. In Germany, however, heating at 100°C for 5 min. is still in general use. Some German mother's milk centres heat the milk at 100°C for 10 min., some at 90°, and e.g. in Munich the practice is 8 min. at 95°C (KAYSER [118]). In 1919—1922 heating at 110°C for 40 min. was used in Magdeburg (SAUER [172]). However, KAYSER [116, 117, 118] has never found any detrimental effects in practice from Holder-Time pasteurization. The mother's milk centres of Kansas City, Missouri, also sterilize breast milk by boiling it for 50 min. (HERVICK [91]). BROADHURST et al. [23] and SCHEUER et al. [175] favour pasteurization at 175°F (89.4°C) for 30 min. on three consecutive days.

LAWS et al. [132] pasteurized breast milk either by the "open-kettle" method ad 165°F (73.9°C) for 30 min. or the "closed-sterilizer" method at 145°F (62.8°C) for 30—35 min., followed by rapid cooling. Both methods produced good results. MACPHERSON et al.[138] pasteurized milk at 140—145°F (60—62.8°C) for 30 min. and cooled it rapidly ("Quick frozen process"). It remained bacteria-free for several months. WRIGHT [205] found that Holder-Time pasteurization gave better results than high pasteurization, reducing the bacteria by 99.9 per cent. SAUER [172] is of the opinion that breast milk must be heated at 100°C for 5 min., lower temperatures being insufficient to kill certain pathogenic bacteria such as E. coli.

The American Academy of Pediatrics [4] issued the following instructions for the operation of mother's milk centres: breast milk must be pasteurized in bottles (each bottle containing as much milk as the baby in question requires daily), in a water bath at 145°F (62.8°C) for 30 min., then cooled in 30 min. to 40°F (4.4°C). The instructions stipulated that the refrigeration temperature was never to be higher than 40°F (4.4°C), not at the donor's home either.

According to CHRISTENSEN [35] Danish mother's milk centres employ pasteurization at 69°C for 10 min., followed by rapid cooling in water, and the amount to be consumed the same day is placed in a refrigerator. The surplus milk is deep-frozen at —8—10°C, as is customary at present almost everywhere in the world (MACPHERSON et al. [138], CATEL et al. [30], WRIGHT et al. [206], LEISTI [135], CHRISTENSEN [35], EMERSON et al. [54], AMERICAN ACADEMY OF PEDIATRICS [4]).

A number of investigators record among the disadvantages of pasteurization the destruction of vitamin C (CATEL et al. [30], MACPHERSON et al. [138], CATEL [31], WOLF [211]). Some consider that the vitamin C content is somewhat reduced, but not due to pasteurization alone — the length of storage also contributes to the loss (HOLMES [96], v. SYDOW [190], CHRISTENSEN [35], KEENEY et al. [119], DYNSKI-KLEIN [53]). KEENEY et al. [119] found that the loss of vitamin C was 20 per cent and attributed it more to pasteurization time than temperature. In addition they found that copper containers and light reduced the content of vitamin C. They found HTST pasteurization preferable in this respect.

The general opinion, however, is that the vitamin C content of both human and cow's milk is too low in any case; hence the milk must be pasteurized in any event and the infants given an additional 25 mg of vitamin C a day. The generally accepted pasteurization methods are HTST pasteurization (heating at 162°F (72.2°C)) for a mininmum of two minutes) and Holder-Time pasteurization (140—150°F (60—65.5°C)) for 30 min.) (KON [127]).

We can well concur with WILSON's [207] conclusion: "Cleanliness of production is no safeguard against tuberculosis or contagious abortion in the cows; and T. T. milk is just as exposed as ungraded milk to infection from human sources. Though the ultimate ideal may be clean milk produced from disease-free herds and protected from human contamination, there is no other immediate practical solution to the problem of supplying safe milk to the public but pasteurization. Whether we like it or not, the logic of the case is inexorable."

ON THE BASES OF ASSESSING THE KEEPING QUALITY OF MILK

A. Practical bacterial study and the bacteriological assessment of milk

a. *Cow's milk*

The problem of hygiene is a problem of storage. Enzymes, bacteria and cells are of importance in judging the quality of milk.

The international practice is to classify cow's milk by the reductase test, which is an enzymatic reaction. A certain amount of methylene blue is added to a certain amount of milk and kept at a temperature of 38°C until decolourisation is observed. According to BARTHEL [9, 10] the primary function of the reductase test is to show the keeping properties of the milk and not directly the bacterial content, although the rate of reduction of methylene blue is always proportionate to the bacterial count of the milk, which can be seen from the classification principles applied in dairy practice (THOMÉ [194], BARTHEL [10], CLARK et al. [36]).

1st class milk: the blue colour persists for over $5\frac{1}{2}$ hours, bacterial count 750,000/ml.

2nd class milk: the blue colour persists for 3—$5\frac{1}{2}$ hours, bacterial count 5 million/ml.

3rd class milk: the blue colour persists for 20 min.—3 hours, bacterial count 4—20 million/ml.

4th class milk: the blue colour persists for less than 20 min., bacterial count over 20 million/ml.

There are milk qualities of which much stricter requirements are made. For instance, Tuberculin-tested and standard milks may contain a maximum of 200,000 bact./ml and certified milk no more than 30,000 bact./ml, etc. (BROOKE [25]). (Further information on the assessment, treatment etc. of cow's milk is obtainable from the publications Report of the Special Milk Board of the Massachusetts State Department of Health [162], and U. S. Public Health Service Ordinance and Code [161].)

RULLMANN et. al. [169] observed that cow's milk with a high leucocyte content also shows a high streptococcus content, evidence of streptococcus mastitis of the cow. BREED [21] again considers the occurrence of polynuclear white blood cells and epithelial cells in cow's milk of no diagnostic significance and holds that pathologic conditions cannot be judged from cells alone. FREI et. al.[70] consider the appearance of leucocytes in milk as the first symptom of a diseased udder.

b. *Mother's milk*

As breast milk intended for general consumption is received by the mother's milk centre it is subjected to qualitative examination e.g. by determining its bacterial content. The maximum permissible bacterial content has varied between 50,000—100,000 bacteria/cc (CHRISTENSEN [37]).

The presence of E. coli bacteria in breast milk is a condition for its rejection (CHRISTENSEN [37]).

DUDGEON et al. [50] found pus (polymorphic leucocytes) in breast milk before the appearance of any clinical symptoms of mastitis. According to them, the occurrence of numerous polynuclears in the milk on the fourth day of lactation is evidence of an infection

which need not manifest itself clinically as mastitis and which usually only sets in later.

Thöni [197] considered milk with a high leucocyte content definitely detrimental to health.

The ''reductase test'' employed with cow's milk is not used in practice for the bacteriological assessment of breast milk.

v. Sydow [190] found that breast milk collected in Gothenburg revealed lower bacterial counts than the dairy milk of the town but never satisfied the New York standard for ''Grade A milk'' (a maximum of 200,000 bact./ml = ordinary commercial milk) or that of commercial milk in Oslo. The same author states that, in the period November-April, 63 out of 202 samples gave a bacterial count exceeding 200,000/ml and 15 over 10 million/ml. He found in samples with a high count mostly bacteria of E. coli type, and in certain cases haemolytic streptococci also. Leisti [186], in milk received at the Turku mother's milk centre, found that only 13 out of 32 samples had a maximum of 200,000 bact./ml, 3 gave a bacterial count of 1,000,000—2,000,000, 7 2,000,000—4,000,000, and one 4,000,000—6,000,000. According to v. Sydow [190] acidity, putrid odour or impurities were by no means always found in the milk samples with the highest bacterial content. Indeed these changes were also found in milk with a low bacterial content. Scherer [174] examined 50 breast milk samples; one fourth had a bacterial count of under 50,000/ml, half the remaining samples had under 500,000 and half over 500,000 bact./ml. In a single sample only was the bacterial content approx. 2,000/ml. The samples containing over 500,000 bact./ml derived partly from mothers who themselves expressed their milk at home or had no adequate storage facilities, partly from mothers sending their milk over longer distances.

The interval between expressing the breast milk and its sterilization is approx. 1—2 days in Helsinki. The high bacterial content of milk is sometimes partly due to this long interval. It is evident, however, that it is not the long storage period alone that is responsible for the infection of the milk. There are donors who always deliver practically sterile milk, e.g. with less than 100 bact./ml (v. Sydow [190]) and 500—900 bact./ml (Laws et al. [133]) in spite of a long storage period while others have milk with a high bacterial

content. Other reasons for this high bacterial content therefore exist. In spite of the fact that the mothers look presentable and the condition of their homes is beyond reproach obviously they are not all of the same high hygienic level as regards their milk, as well-kept cows. However, it should be borne in mind that breast milk may be a better culture medium than cow's milk, perhaps due to the high sugar content of breast milk (v. SYDOW [190]).

B. Odour and flavour of milk

a. *Cow's milk*

The bacteria entering milk induce fermentation and decomposition phenomena involving in part lactose, in part fats and proteins. These processes also lead to a change in the flavour of the milk produced by the fermentation and decomposition products. BROCK [24] maintains that the best criterion by which to judge milk is its flavour.

Everybody knows the taste of cow's milk. It is rich, fairly sweet.

ROADHOUSE et al. [164], studying the taste of milk, found that a high chloride and lactose content detracts from the flavour of milk. They describe the normal flavour of milk as the primary taste and are of the opinion that it is milk dialysate that contains the taste properties and that fat and proteins are not of equal importance to the flavour. "The primary taste of milk has been designated as the sum of one of the taste impressions coming from normal milk and not influenced by feed or the secretion of abnormal milk."

The rancid flavour in cow's milk is produced by Corynebacterium bovis, and by putrefying bacteria, e.g. Pseudomonas fluorescens and Serratia marcescens, which are capable of decomposing milk fat into its components. The fatdecomposing bacteria of the saprophytes group that enter milk temporarily are responsible for the soapy and fishy flavours found in milk (GRIMMER [81]).

Butyric acid bacteria, emanating from cow manure, turn lactose (not milk fat) primarily into butyric acid. As they are anaerobic they develop when the access of oxygen to the milk is inhibited

(high milk cans). Butyric acid fermentation produces in the milk a putrid smell and flavour which makes the milk unfit for use (FEER [59], BARTHEL [10]).

Peptonizing bacteria are responsible for the bitter taste of milk. Poor contact between the air and the milk first leads to the development of a sharp odour and taste, then a putrid odour and taste. The peptonizing bacteria — hay bacteria — are sporulating and derive from the cow's food, expressly hay. These bacteria do not produce fermentation, i.e. decomposition of carbohydrates, but decomposition of proteins which almost corresponds to the digestion of food (peptonization). In bitter milk casein has undergone advanced decomposition, i.e. the result is peptones characterized by a very bitter taste (FEER [59], GRIMMER [81], etc.).

Bacillus lactis saponacei is responsible for the soapy flavour of cow's milk. It is present in second-quality hay, in fodder and in linseed meal (GRIMMER [81]).

GOULD et al. [80] have found that the cooked flavour of milk is due to sulphides formed when milk is brought to a high enough temperature. ROADHOUSE [165] found that the metals of the pasteurization plant, especially copper, may impair the flavour of milk. JOSEPHSON et al. [114], again, found that copper eliminated the cooked flavour of milk by inhibiting the formation of the sulfhydryl group. They are of the opinion that all the components responsible for taste are oxidizable substances and that the cooked taste may derive from denaturation due to heat of one or more proteins, in the first place lactalbumin and the protein constituent of the fat globule membrane. EMERSON et al.[54] point out that various fodders produce a poor taste in cow's milk unless used in reasonable quantities immediately after milking.

However, the flavour does not always indicate decomposition or the extent of decomposition in milk. For instance, the milk may contain enormous quantities of E. coli bacteria before fermentation begins. First the bacteria proliferate; their decomposition follows, and ferments, responsible for the decomposition process, are only liberated from the decomposed bacteria. Hence even milk with an entirely satisfactory flavour and odour contains enormous quantities of living organisms promoting decomposition (FLÜGGE [67], GOTSCHLICH [79], BARTHEL [10]).

b. *Mother's milk*

Breast milk is very sweet due to its high lactose content.

The ordinary lactic acid bacteria present in milk, lactococci and lactobacilli, in conditions favourable to them produce by lactose fermentation plenty of the lactic acid responsible for the well-known sour taste of milk. If breast milk is sour on reaching the mother's milk centre this indicates that the bacterial growth is abundant and that the milk has not been handled carefully enough (CHRISTENSEN [35]).

WRIGHT [204], speaking of mother's milk, says: ''The high fat content of 'strippings' from the breast and the easy development of rancidity in human milk have been obstacles to good keeping quality.''

An essential condition therefore is that the milk to be used has no flavour defect but, as stated above, faultless flavour is no guarantee of the quality of the milk.

C. Investigations of the pH of milk

Both fresh mother's milk and cow's milk show an alkaline reaction to litmus but an acid reaction to phenolphthalein. A high alkalinity is required to produce a neutral reaction to phenolphthalein. The alkaline quantity depends on the titre of acidity. In titration according to Soxhlet-Henkel with n/4 alkali the neutralization of milk requires 7 cc of alkali per 100 cc of cow's milk. This titratable ''titre of acidity'' is not the same as the H·-concentration of lactic acid for it includes the dissociated H ions of the acid salts, casein, carbonic acid and possibly also of amino acids, and pH is not comparable with the above-mentioned titratable acidity (FREI et al. [70], SCHERER [174], WEDEMANN [199], PESCH et al. [157], OBERMEIER [153]).

The pH of breast milk varies between 6.58—7.21, with 6.97 as the mean value (DAVIDSOHN [41], ARON [7]), while the pH of cow's milk varies between approx. 6.40—6.70 (FREI et al. [70]).

SZILI [191] obtained a pH value of 7.3 for breast milk on the first day of lactation. KAYSER's [118] pH value for breast milk was 7.2—7.6.

The addition of water and removal of cream do not change the pH value. Similarly cooking has scarcely any effect on the pH value (FREI et al. [70], OBERMEIER [153]), though SELESTE [181] has observed a slight rise in pH after heating.

FREI et al. [70], PESCH et al. [157], BAKER et al. [8] and OBERMEIER [153], in the initial stages of udder inflammation, have found a slightly raised pH value, caused by the entry into the milk of leucocytes and other components of blood and possibly by protein-decomposing, alkali-forming bacteria.

The pH of cow's milk is independent of pregnancy and rut, of the age of the animal and of food. The pH value of colostrum and its whey, under 5.3 on the day of parturition, is lower than that of normal cow's milk. From the third postpartum day onwards the pH values are fairly evenly higher (FREI et al. [70]). These researchers made their tests with bromcresol purple and bromthymol blue and, in determining the pH of whey, treated the whey with chlorinated lime, obtaining somewhat lower values than those obtained by electrometric investigation (pH 5.9—6.1). In addition, they showed that the pH value of whey is subject to an average individual variation of 0.39; the daily fluctuations vary between 0—0.6, depending on the herd and the animal, a variation that diminishes slightly towards the end of lactation. In milk taken from any one udder quarter, at rest in the morning, the pH of the first milk drawn is lower than that of the last, the difference being 0.1—0.2. At night the results are generally the opposite.

Souring is a biochemical phenomenon. Lactic acid produced by lactic acid bacteria continuously changes the ion equilibrium of the milk; the change is the increase in H ions (FREI et al. [70]). No wonder pH fluctuations are studied so closely in judging milk.

However, HOLWERDA [98] says that ''— weder die erreichte H-Ionenkonzentration in irgendeinem Medium noch der potentielle Säuregrad ein biologisches Merkmal der Milchsäurebakterien sein kann''.

PESCH et al. [157] again consider it very important in judging the freshness of milk to measure the $H^{\cdot}$ concentration, and they hold that the pH value of milk indicates the stage of decomposition of the milk since its keeping quality is dependent on its buffering ability, i.e. the secondary phosphate: primary phosphate ratio expressed by the pH value.

Cow's milk has a high buffering ability, 2—4 times higher than that of breast milk (GERSTLEY [76], DEMUTH [42], ARON [7], MÜLLER [150]). Naturally this also affects spontaneous milk fermentation (MÜLLER [149], ARON [7]).

FREI et al. [70] kept cow's milk in an open dish at room temperature and investigated the titratable acidity and pH values. Their results were as follows:

Hour	Titre of Acidity	Increase in titratable acidity		pH	Decrease in pH	
		absolute	percentage		absolute	percentage
0.	6.6	—	—	6.0	—	—
4.	6.8	—	—	6.0	—	—
6.	7.0	0.2	2.9	6.0	—	—
21.	7.6	0.8	11.7	5.9	0.1	1.7
23.	8.8	2.0	29.3	5.8	0.1	3.4
26.	13.0	6.2	91.2	5.3	6.7	11.6
30.	17.4	10.6	155.8	4.8	1.2	27.3
46.	26.4	19.6	288.2	4.8	1.2	27.3

Similar investigations have also been made by OBERMEIER [153].

In their detailed study of pH and bacteria PESCH et al. [157] found that the determination of H˙ concentration is very important in judging milk and especially its freshness but that the pH value and bacterial count are in no way correlated nor can the bacterial count be concluded from the pH value. SCHULTZ et al. [178] recommend the determination of the pH value of commercial milk instead of taking the bacterial count. They found that the number of H ions rises with the bacterial count, although irregularly. They studied the bacterial content and actual reaction in the pH area 6.8—4.4. The bacterial count increased continuously ad pH 5.7, but after that they found irregularities which they could not explain. They assumed the irregularities to be due to the presence of phosphoric and lactic acids which adversely affect the metabolism and reproduction of bacteria. In addition, they found that even small increases in the H˙ concentration showed in comparative experiments immense increases in the bacterial count. From their investigations they concluded that the quality of commercial milk can well be judged from pH determinations.

Schultz et al.[178] studied 20 samples per pH value, taking the average bacterial count. The following is an illustrative selection from their numerous figures.

pH	Highest bacterial count	Lowest bacterial count	Average bacterial count
6.8	110,000	750	27,500
6.5	325,000,000	2,500,000	68,000,000
6.2	2,000,000,000	2,000,000	174,000,000
5.9	1,000,000,000	10,000,000	260,000,000
5.6	500,000,000	6,000,000	221,000,000
5.3	800,000,000	4,000,000	280,000,000
5.0	1,500,000,000	6,000,000	384,000,000
4.7	600,000,000	4,000,000	222,000,000

The determination of the actual acidity originates from the generally known biological fact that in chemical and particularly in biological phenomena, in so far as they are connected with acidity or alkalinity, it is not the amount of titratable acid or alkali but rather the $H^{\cdot}$ concentration, i.e. the amount of dissociated or free H or OH ions, respectively, present in the solution that is decisive. As the souring of milk also is a chemico-microbiological phenomenon actual reaction is preferable to the acid titration value, i.e. the acidity of milk according to Soxhlet-Henkel (Pesch et al.[157], Frei et al.[70]), for determining the state of freshness.

Flügge[67] showed in 1894 that peptonizing bacteria either alkalinize the cow's milk or do not affect the reaction at all. Frei et al.[70] considered any increase above the normal in the pH value of milk or whey to be pathological.

Scherer[174] showed that the bacterial content of breast milk could not be assessed from its titratable acidity or from the pH value. He found that unfavourable temperatures, even in prolonged storage, did not essentially affect the pH value and bacterial content of sterilized (100°C — 5 min.) breast milk.

IV. PRESENT INVESTIGATIONS

MATERIAL AND METHOD

To obtain some idea of the keeping properties of milk the author has stored treated and untreated milk at different temperatures and observed its spoiling by tasting and by taking the pH. The flavour has been graded according to the HELSKE [89] method. In addition, a ''superinfection test'' (inoculation test) has been made by actively infecting the milk samples.

The samples of cow's milk required for the investigation were obtained from Rosenlund Farm, Hanala, in Helsinki Rural District. The samples of mother's milk were obtained partly from the donors supplying the Children's Clinic and ''Maitopisara'' mother's milk centres and partly from the wet nurses at the Children's Clinic. The mothers gave their milk at the place of investigation and the cow's milk was brought in immediately after milking. Both cow's milk and mother's milk were taken with the most careful attention to asepsis. The breasts and the udders were first cleaned with 96 per cent ethyl alcohol, on which the milk was milked into a sterile container.

Immediately on receipt of the samples some of the milk was placed in a sterile Erlenmeyer flask, sealed with a cork or rubber plug with a hole in the middle to take a thermometer, and heated in a water bath to 62°C. The milk was kept at 62°C for 20 min. (Holder-Time pasteurization). The flask was carefully shaken all the time to ensure even heating. After this the milk was rapidly cooled in running water, and equal quantities were measured out into small Erlenmeyer flasks, the entire operation being conducted in strictly sterile conditions. Another sample of the milk in a similar flask was heated in the same way in a water bath to 80°C (HTST pasteurization), kept

there for 2 min., rapidly cooled in running water and distributed into small flasks. The balance of the milk, not heat-treated, was cooled in running water and then poured into similar small flasks. During the investigations these flasks, in which the milk samples remained for 5 days, were kept sealed and only opened to take the quantities required for pH determination. The pH determinations and tasting were carried out immediately the samples arrived and after pasteurization at 9 in the morning, and subsequently regularly at 3 p.m. and 9 a.m. for five days. Each of the three series consisted of 20 different milk samples. The pH determinations were effected by a pH meter fitted with a glass electrode (Radiometer M11).

For the "superinfection test" (inoculation test), Streptococcus lactis pure culture grown on sterile cow's milk, from the Biochemical Research Institute, was added to the samples. The additions consisted of 0.5 cc:100 cc for mother's milk and 1 cc:100 cc for cow's milk, according to STORGÅRDS[187] who considered that these ratios correspond to one another. The third series received a similar addition of E. coli (bacterial strain 85993) grown on sterile cow's milk, obtained from the University Department for Serology and Bacteriology.

No taste tests were made of the series inoculated with E. coli bacteria; the other two series were subjected to tasting.

No method of grading flavour nuances was found in the literature. Before embarking on the present investigation the author made taste tests of various solutions in different concentrations in order to obtain an objective standard. The over 20 flavour nuances employed in the original tests are classified as three basic flavours:

5. sweet (watery sweet, sweetish, fat sweet)
4. turned (bitter sweet, watery bitter sweet, watery)

sour:

3. sourish (sweet sour, sweet sourish, sweet rancid, watery sweet sourish, bitter sweetish, etc.)
2. sour (sharply sourish, watery bitter sour, bitter sour. etc.)
1. vinegar sour (sharply sour, bitter sour, spoiled, rancid etc.)

As it can be assumed that every observer means approximately the same in speaking of sweet milk and milk that has turned, these were chosen as the basic flavours. Nuances 3, 2 and 1, collectively, are termed sour, the term used for all the samples with a flavour that makes the milk undrinkable.

In order to obtain an objective mean value for the flavours the following procedure was adopted.

If we take it that, at a certain point of time, m milk samples out of 20 are sweet, n samples turned and k samples sour, $m + n + k = 20$. If sweet samples total 15 ($= 75$ per cent) or more, the mean value entered is sweet; if again sweet samples are less than 15 and the aggregate number of sweet and turned is 15 or more, the mean value entered is turned; in the other cases the mean value entered is sour. Schematically this can be represented as follows:

If $m \geqq 15$, the mean value is sweet;
if $m < 15$, but $m + n \geqq 15$ the value is turned;
if $m + n < 15$, the mean value is sour.

Let us assume that the table of test results is as follows:

		sweet	turned	sour
1st day at	9 a.m.	20	0	0
	3 p.m.	18	2	0
2nd day at	9 a.m.	17	2	1
	3 p.m.	14	3	3
3rd day at	9 a.m.	10	6	4
	3 p.m.	9	6	5
4th day at	9 a.m.	7	5	8
	3 p.m.	3	4	13
5th day at	9 a.m.	2	3	15
	3 p.m.	0	1	19

It is found that the mean value remains sweet until 9 a.m. on the second day, when sweet samples still total 17. At 3 p.m. the total of sweet samples is only 14, but the sum of sweet and turned exceeds 15, viz. $14 + 3 = 17$; hence the mean value is turned.

This mean value persists until 3 p.m. on the third day, when the sum of sweet and turned is $9 + 6 = 15$, but on the fourth day, at 9 a.m., this sum is only $7 + 5 = 12$; hence the mean value is sour and remains sour throughout the final period of the experiment.

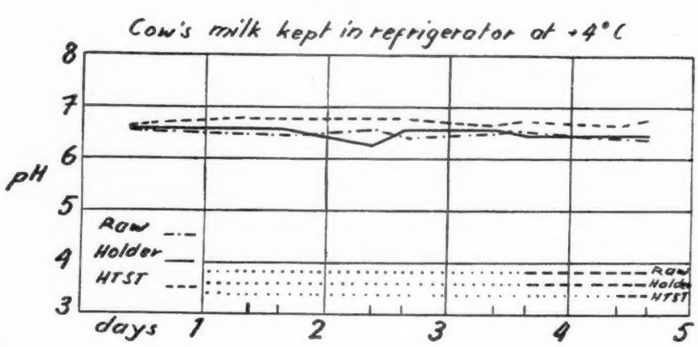
KEEPING QUALITY OF RAW AND PASTEURIZED MILKS WITH REFERENCE TO pH AND FLAVOUR CHANGES

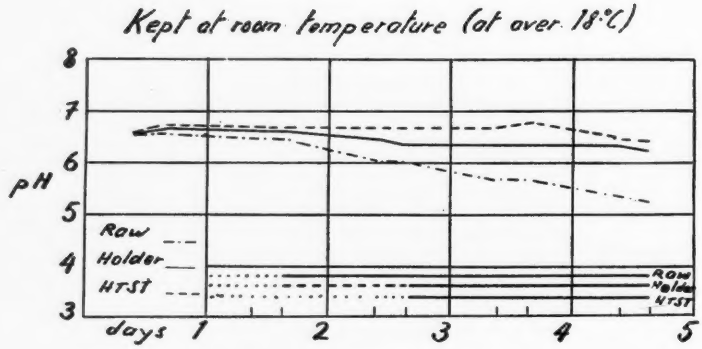
TESTS WITHOUT INOCULATION

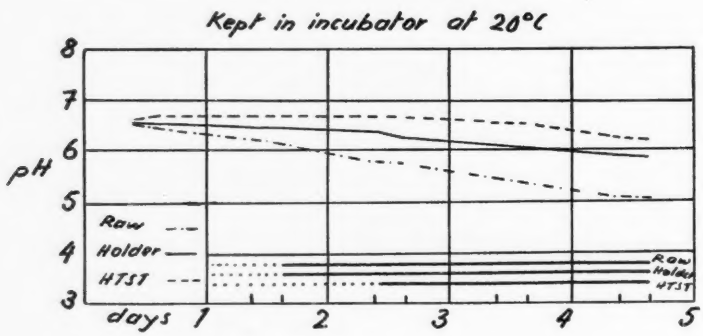
A. Cow's milk

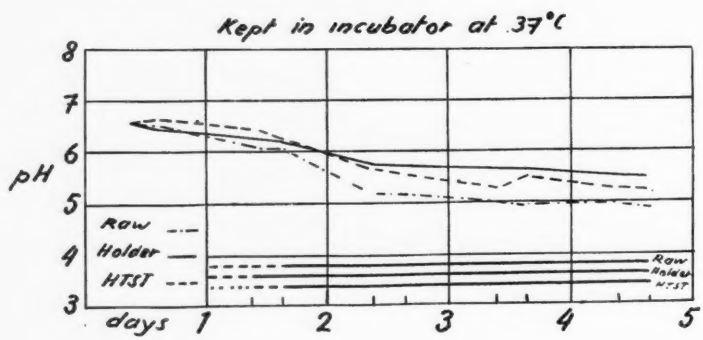
Graph I shows the variations in the pH and flavour of cow's milk, raw, Holder-Time pasteurized (62°C — 20 min.) and HTST pasteurized (80°C — 2 min.), kept 5 days in a refrigerator, at room temperature, at 20°C and at 37°C. The symbols indicating the flavour are inserted in the bottom part of each graph in the same order as the milk samples are listed in the square in the left-hand corner.

Graph I.









sweet ....
turned - - - - -
sour ———

Review of Results: The curves show that HTST pasteurized cow's milk retains its flavour best at all temperatures while raw and Holder-Time pasteurized cow's milk undergo parallel flavour changes.

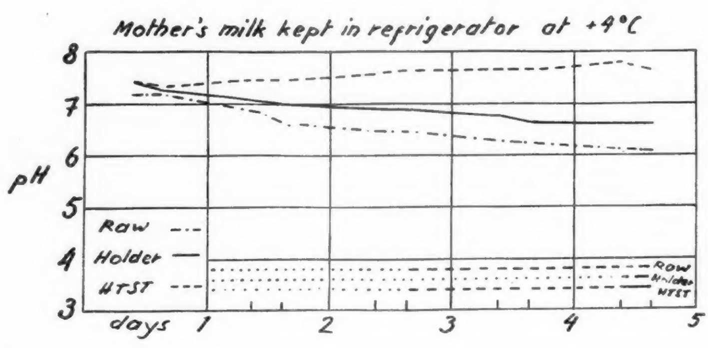
As a rule the pH variations also are smallest in HTST pasteurized cow's milk. The differences from other cow's milk samples emerge best on storage at room temperature and at + 20°C. Only at 37°C do the pH values of HTST pasteurized cow's milk show a greater drop than those of Holder-Time pasteurized. The pH curves of raw cow's milk, apart from the refrigerator curve, take a definite course towards acidity.

A study of the pH and flavour reveals further that a normal or approximately normal pH value is not always concurrent with a good flavour. For instance, kept at room temperature the pH of HTST pasteurized cow's milk on the afternoon of the 5th day was 6.55, its flavour sour.

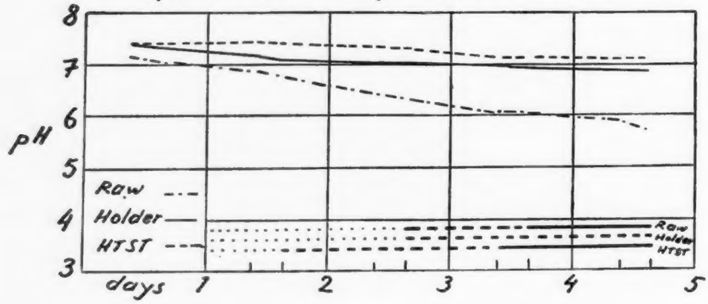
B. Mother's milk

Graph II shows the variations in the pH value and flavour of raw, Holder-Time pasteurized and HTST pasteurized breast milk, kept and treated in the same way and in the same conditions as described for cow's milk above.

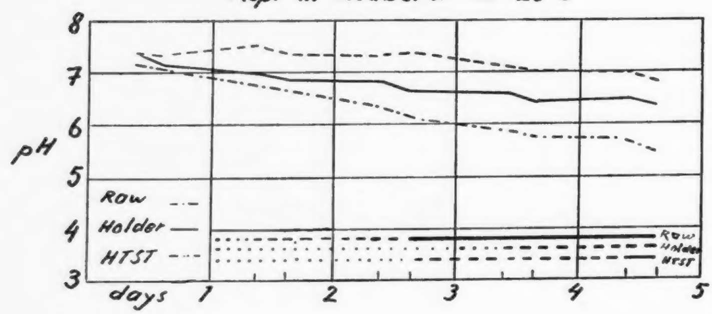
Graph II.



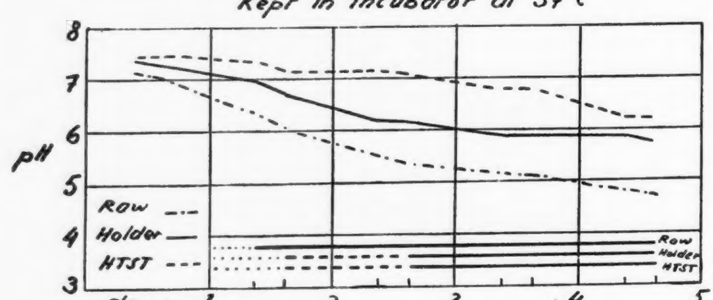
Kept at room temperature (at aver. 18°C)



Kept in incubator at 20°C



Kept in incubator at 37°C



sweet ....
turned - - - - -
sour ———

Holder-Time pasteurized breast milk retains its flavour best at all temperatures except 37°C. At this temperature HTST

pasteurized breast milk remains similar in flavour to Holder-Time pasteurized. This is the more remarkable as, according to the pH curves, Holder-Time pasteurized breast milk samples increase in acidity more than the HTST pasteurized although not as much as raw breast milk. As regards flavour HTST pasteurized breast milk is inferior to raw milk at the lower temperatures but superior at the higher. It is in the pH curves that these milk samples differ most from one another, the raw milk always towards the sour side while HTST pasteurized breast milk kept in a refrigerator tends to assume a pH value indicative of alkalinity even. With temperatures rising the pH generally drops.

If we study the pH value and flavour we find, in addition, that not even in breast milk does a normal or approximately normal pH value always go with good flavour. For instance, the pH of HTST pasteurized breast milk kept in a refrigerator is 7.73 on the afternoon of the 5th day; in spite of this the flavour is sour!

C. Comparison of cow's milk and mother's milk

Graphs I and II show that cow's milk and breast milk react slightly differently at identical storage temperatures. It must be borne in mind that the starting point for breast milk pH values is considerably higher than that of cow's milk, and the fluctuations are greater than in cow's milk. To illustrate the point the quality of each milk sample has been graded 1—3, with 1 representing the best and 3 the poorest quality; the grading can be seen from the following table.

	Flavour				pH			
	+4°	18°	20°	37°	+4°	18°	20°	37°
Cow's milk								
Raw	2.5	3	2.5	2	2	3	3	3
Holder-Time pasteurized	2.5	2	2.5	3	2	2	2	1
HTST pasteurized	1	1	1	1	2	1	1	2
Mother's milk								
Raw	2	3	3	3	3	3	3	2
Holder-Time pasteurized	1	1	1	2	2 acid.	2	2	2
HTST pasteurized	3	2	2	2	2 alk.	1	1	1

The results show that the best method of keeping cow's milk, at least as far as flavour is concerned, is HTST pasteurization. Nor do the pH results contradict this finding.

For breast milk Holder-Time pasteurization is definitely the best as far as flavour goes. Judging by the pH curves the result is not the same — the changes in HTST pasteurized milk are smaller — but in view of the marked alkali formation in HTST pasteurized breast milk the changes in pH are probably not very indicative. The advantages of Holder-Time pasteurization for breast milk are more distinctly seen from the results of storage at refrigerator temperatures. Hence Holder-Time pasteurization seems the most advantageous method of keeping breast milk.

"SUPERINFECTION TESTS" INOCULATION TESTS)

As mentioned before (p. 33) one way of obtaining an idea of the keeping properties of milk is to infect the milk (Storgårds [187]). The present investigations into this method are reported on below.

I. Secondary inoculation: Str. lactis

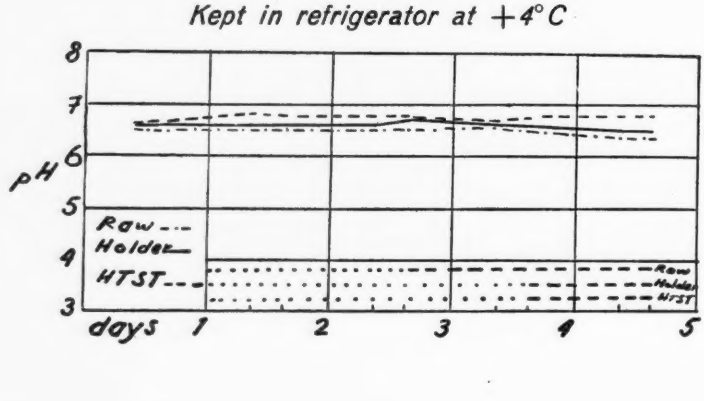
A. *Cow's milk*

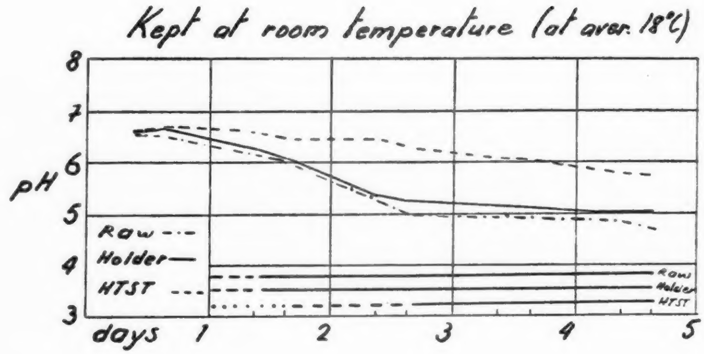
Graph III illustrates the same samples of cow's milk as Graph I, but in Graph III Str. lactis has been added.

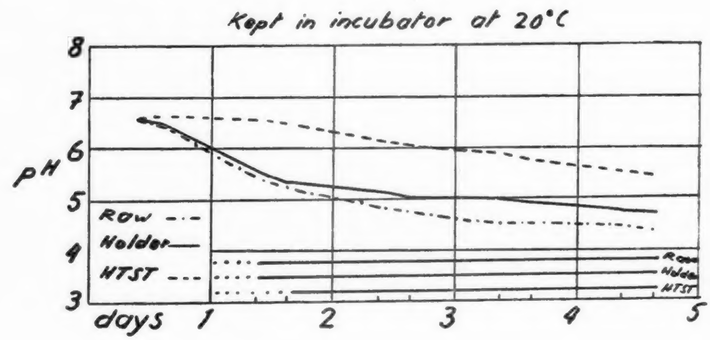
Graph III.

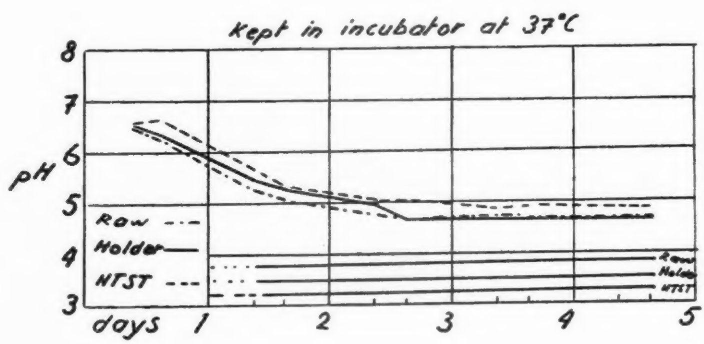
Cow's milk inoculated with str.lactis

Kept in refrigerator at +4°C









sweet ....
turned - - - - -
sour ———

Review of results: The graphs show that as far as pH variations are concerned it is only at refrigerator temperature that milk does not sour to any considerable extent. However, inoculated milk

perhaps sours slightly more rapidly than the corresponding samples without bacteria added.

The differences between inoculated and uninoculated cow's milk samples emerge more distinctly at higher temperatures. The former reveal greater pH changes towards the sour side than the latter. But even in the inoculated samples the pH changes are smallest in HTST pasteurized milk and greatest in raw milk except at 37°C. At this temperature the final pH values of raw milk are slightly higher than those of Holder-Time pasteurized, although initially raw milk sours most rapidly.

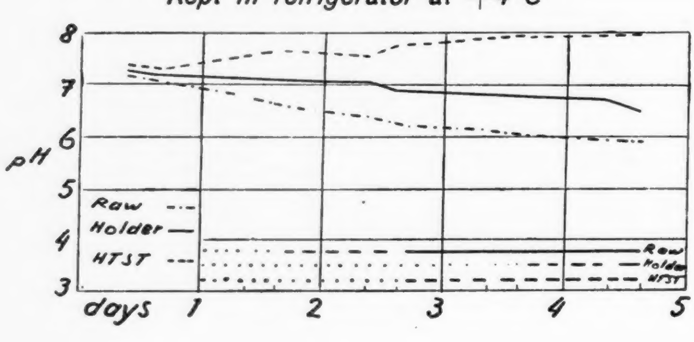
As regards flavours, HTST pasteurized milk retains its sweet flavour slightly longer than the other samples, and as a rule longer than raw milk. These flavour differences are most marked at relatively low temperatures. The same phenomenon is noticeable in the corresponding cow's milk samples to which no Str.lactis was added. HTST pasteurized cow's milk inoculated with Str.lactis retains its good flavour longer than the corresponding Holder-Time pasteurized milk; the latter again keeps its flavour somewhat better than raw milk at a low temperature (refrigerator temperature). At higher temperatures there are no flavour differences between Holder-Time pasteurized and raw cow's milk.

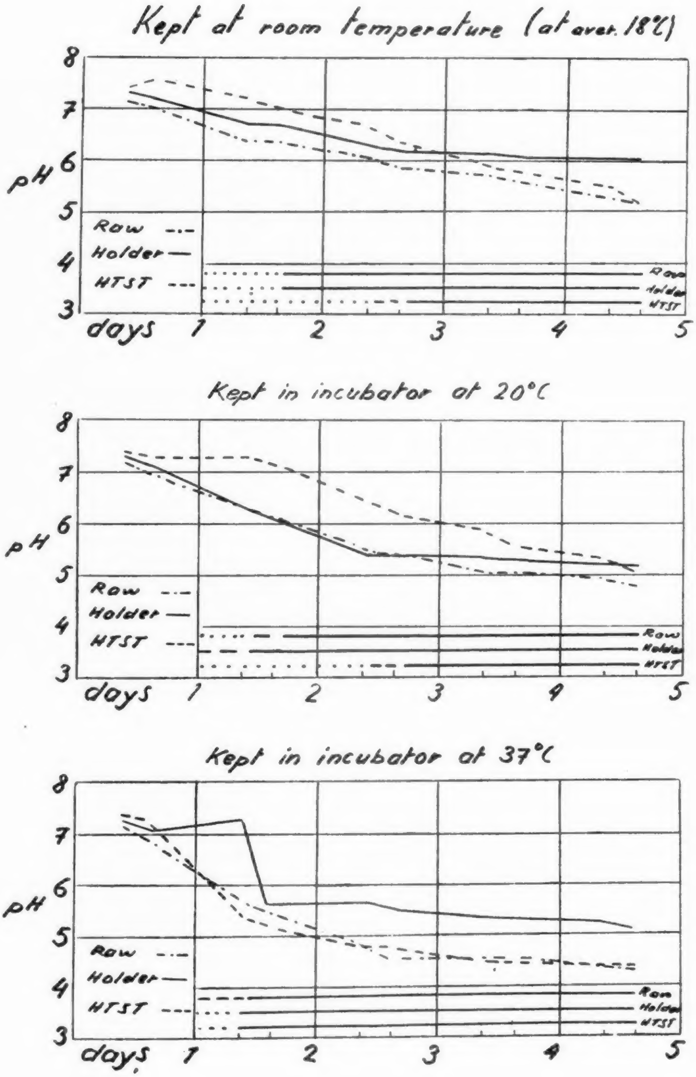
B. *Mother's milk*

Graph IV illustrates the same breast milk samples as Graph II, except that Str.lactis has now been added.

Graph IV.

Mother's milk inoculated with str.lactis





sweet ....
turned - - - - -
sour ————

Review of results: The graphs show that as far as pH variations are concerned there is no souring worth mentioning at refrigerator

temperature in breast milks inoculated with Str.lactis, no more than in the corresponding cow's milk samples. However, the souring is slightly more marked than in uninoculated breast milk samples except HTST pasteurized breast milk in which the pH values change towards greater alkalinity.

The difference between inoculated and uninoculated breast milk samples are more distinct at higher temperatures. Str.lactis samples reveal considerably greater pH variations towards the sour side than the uninoculated samples in all the breast milks. Raw ''Str.lactis breast milk'' reveals greater pH changes towards the sour side than pasteurized ''Str.lactis breast milk'' samples at all temperatures, whereas the difference between Holder-Time pasteurized and HTST pasteurized ''Str.lactis breast milk'' samples diminishes at the higher temperatures; however, the Holder-Time pasteurized ''Str.lactis breast milk'' as a rule varies slightly less than the HTST pasteurized ''Str.lactis breast milk'', although variations exist to one side or the other.

The difference between Holder-Time pasteurized and HTST pasteurized ''str.lactis breast milk'' samples is most distinct at refrigerator temperature: the former reveals an uninterrupted, slight souring tendency, the latter becomes increasingly alkaline — even more so than the corresponding uninoculated milk.

In these inoculated samples HTST pasteurized breast milk generally retains its good flavour longer at the higher temperatures than Holder-Time pasteurized breast milk; the latter, again, remains sweet longer than raw breast milk. At refrigerator temperature only is Holder-Time pasteurized breast milk best in its flavourkeeping quality.

A study of the flavour graphs shows further that the breast milk samples inoculated with Str.lactis reveal flavour changes at higher temperatures very similar to those in uninoculated cow's milk samples.

If, in addition, we compare the pH variations and flavour changes of inoculated cow's milk with inoculated breast milk samples we can see that, as with the uninoculated milk samples, a normal or fairly normal pH is not always indicative of good flavour.

C. *Comparison of cow's milk and mother's milk*

Graphs III and IV show that even with Str.lactis added cow's milk and breast milk react slightly differently at identical temperatures. To illustrate the point, as with Graph I and Graph II (p. 39), the quality of the milk samples has been graded 1—3. The figures are as follows:

Str.lactis	Flavour				pH			
	+4°	18°	20°	37°	+4°	18°	20°	37°
Cow's milk								
Raw	2	2.5	2.5	1.5	3	3	3	2.5
Holder-Time pasteurized	1.5	2.5	2.5	1.5	2	2	2	2.5
HTST pasteurized	1.5	1	1	3	1	1	1	1
Mother's milk								
Raw	2	2.5	2.5	1.5	3	3	3	2.5
Holder-Time pasteurized	1	2.5	3	1.5	1 acid	1	2	1
HTST pasteurized	2	1	1	1.5	2 alk.	2	1	2.5

It is found that Str.lactis causes the smallest changes in pH and flavour in HTST pasteurized cow's milk and the greatest in raw cow's and breast milk.

As a rule, Str.lactis affects breast milk more than cow's milk.

HTST pasteurized breast milk inoculated with Str.lactis retains its good flavour best except at a low temperature; at the higher temperatures its flavour changes are very similar to those of cow's milk at similar temperatures. Holder-Time pasteurized breast milk is best with regard to pH changes, better than HTST pasteurized. Bearing in mind the intense alkali formation in HTST pasteurized breast milk at refrigerator temperature and comparing the pH values of this milk with the pH of the same milk kept at higher temperatures, it is in this milk that the greatest drop in pH occurs compared with other corresponding milk samples; it indicates that breast milk suffers most from HTST pasteurization.

The pH variations of breast milk at higher temperatures are less regular than those of cow's milk, in which the souring advances fairly evenly.

II. Secondary inoculation: E. coli

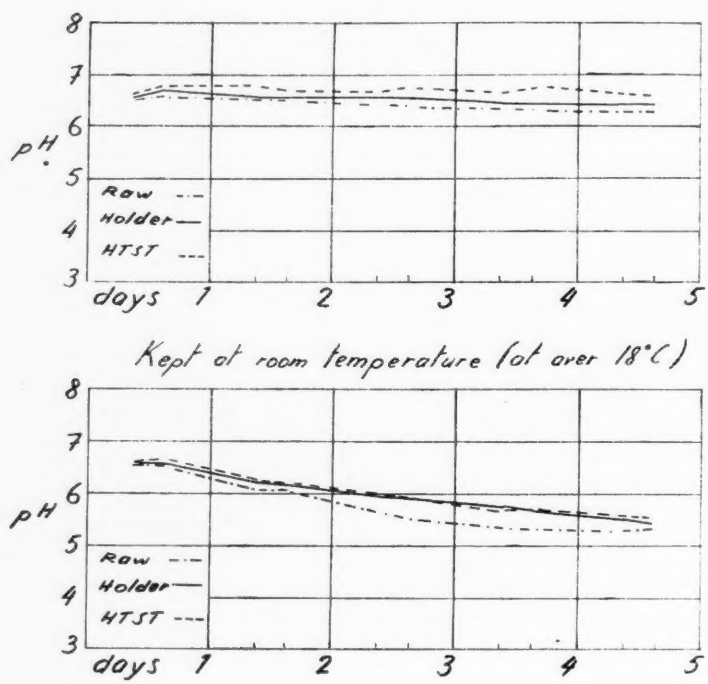
A. *Cow's milk*

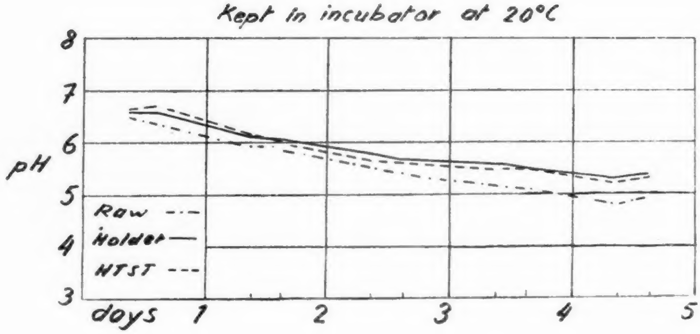
Graph V illustrates the same cow's milk samples as Graph III, the only difference being that E. coli has been added instead of Str. lactis.

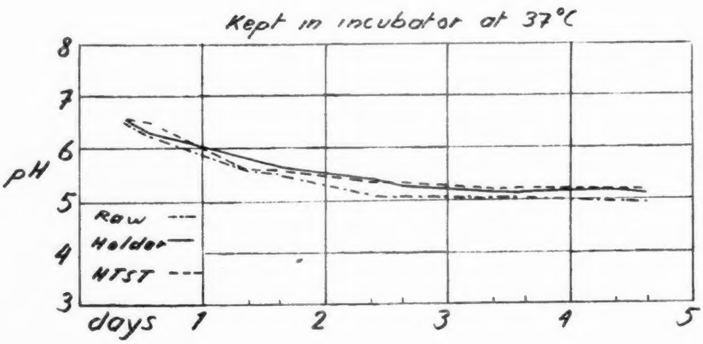
Graph V.

Cow's milk inoculated with E. coli

Kept in refrigerator at +4°C







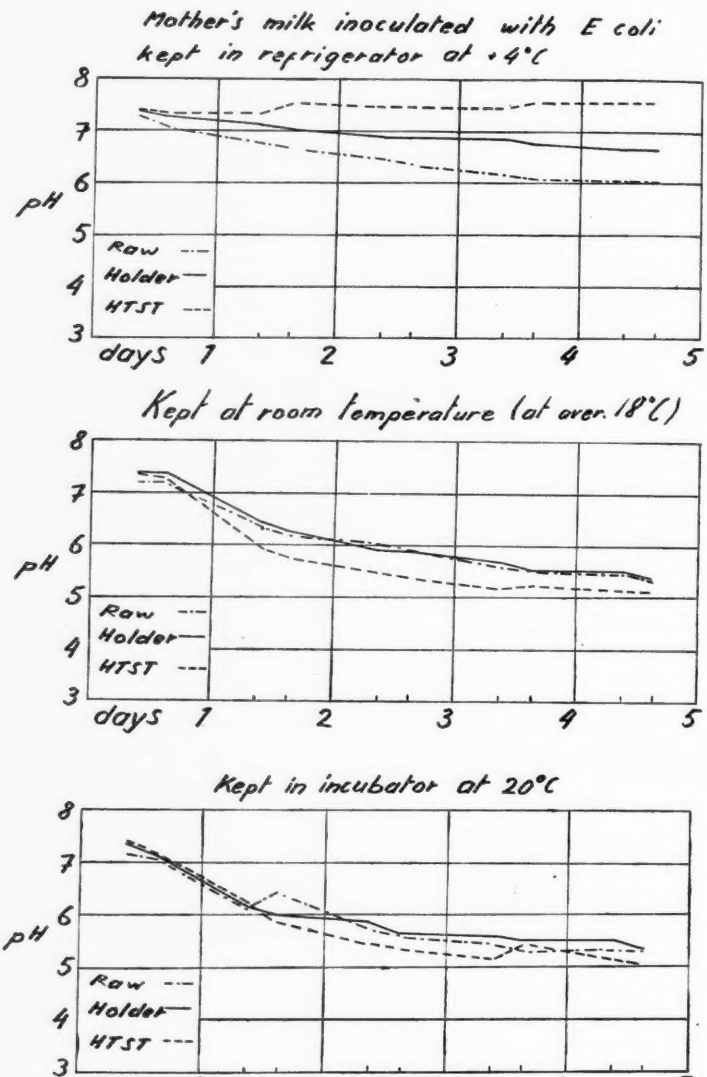
Review of results: The pH variations at refrigerator temperature in cow's milk inoculated with E.coli do not differ much from those found in the corresponding cow's milk samples with no bacteria added or inoculated with Str.lactis. The samples inoculated with E.coli sour slightly more than the others.

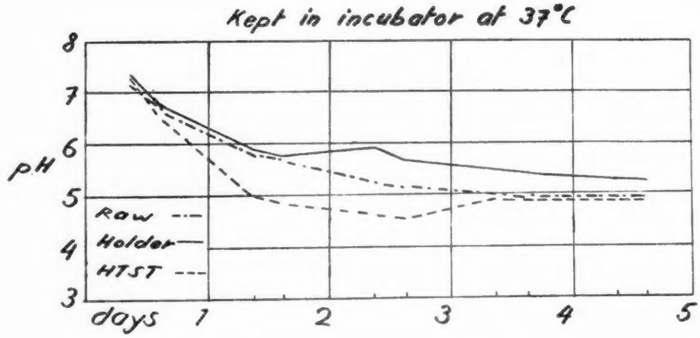
At higher temperatures raw cow's milk inoculated with E.coli shows the greatest variations towards the sour side, though not as great as those induced by Str.lactis. The pH variations of pasteurized cow's milk assume a practically parallel and continous course towards the sour side; however, the pH variations in HTST pasteurized milk are slightly smaller than those of Holder-Time pasteurized milk.

B. *Mother's milk*

Graph VI illustrates the same mother's milk samples as Graph IV, the only difference being that E. c o l i has been added instead of Str.lactis.

Graph VI.





Kept at refrigerator temperature, the pH variations of Holder-Time pasteurized and raw breast milk differ very little from the pH variations present in the corresponding breast milk samples without a bacterial addition and those inoculated with Str. lactis. HTST pasteurized breast milk, on the other hand, reveals the greatest differences, with pH values decreasing.

At the higher temperatures HTST pasteurized breast milk sours most, Holder-Time pasteurized least. At room temperature the pH values of raw and Holder-Time pasteurized breast milk fall in much the same way, the Holder-Time pasteurized milk slightly less.

Compared with the pH variations in uninoculated breast milk and Str.lactis breast milk, HTST pasteurized milk reveals the greatest variations towards the sour side. Except at room temperature the drop in pH is generally slightly less after E.coli than after Str.lactis inoculation.

C. *Comparison of cow's milk and mother's milk*

Graphs V and VI show that the addition of E.coli also induces in cow's milk and breast milk a slightly different reaction at identical temperatures. To illustrate the point, as with the previous graphs, the quality of each milk sample has been graded 1—3 in order of superiority, as follows:

E. coli	+ 4°	18°	pH 20°	37°
Cow's milk				
Raw	3	3	3	3
Holder-Time pasteurized	2	2	1.5	2
HTST pasteurized	1	1	1.5	1
Mother's milk				
Raw	3	2	2	2
Holder-Time pasteurized	2	1	1	1
HTST pasteurized	1	3	3	3

E.coli affects HTST pasteurized cow's milk least and raw cow's milk most. With mother's milk the pH changes produced are smallest in the Holder-Time pasteurized samples and greatest in the HTST pasteurized. However, the general course of the variations in both milks is very similar, in contrast to the previous pairs of graphs.

Summary

No absolute correlation exists between the flavour and pH of uninoculated milks.

In the system inoculated with Str.lactis no absolute correlation exists between the flavour and pH of the inoculated milk samples.

Keeping the various kinds of milk at different temperatures gives the following general course of pH variations: pH values change least in the HTST pasteurized, uninoculated cow's milk samples and most in the raw cow's milk samples. Holder-Time pasteurized, uninoculated breast milk samples and the corresponding HTST pasteurized cow's milk samples retain their flavour best.

We can also see from the foregoing that in the uninoculated series, from the point of view of flavour and pH variations, cow's milk is best when HTST pasteurized, and breast milk perhaps when Holder-Time pasteurized. The differences between HTST pasteurized and Holder-Time pasteurized milk are greatest and most distinct at refrigerator and room temperature. At the highest temperatures all raw milk samples, both breast and cow's, show poorer keeping qualities than pasteurized milk.

In addition, in this inoculated system the general course of the pH variations was found to be one of least change in HTST pasteurized cow's milk and greatest change in raw milks. The differences in pH variations in pasteurized breast milks are relatively small and, in a way, smallest in Holder-Time pasteurized breast milk. As a rule, HTST pasteurized milks retain their flavour best; at refrigerator temperature only is Holder-Time pasteurized breast milk best in this respect.

Hence it can be seen from the foregoing that of the milk samples inoculated with Str.lactis the HTST pasteurized generally keep best, except at a low temperature when Holder-Time pasteurized breast milk is best. The greatest differences between the pasteurized milks are most distinct at a low temperature while at higher temperature the pasteurized breast milks reveal pH variations and flavour changes largely similar to those of the corresponding cow's milks and also to those of the uninoculated cow's milks, i.e. breast milk at higher temperatures, possibly due to the Str.lactis addition, becomes similar in character to cow's milk.

In milk inoculated with E.coli the general course of the pH variations is: the variations are smallest in HTST pasteurized cow's milk and greater in raw cow's milk than in Holder-Time pasteurized cow's milk. The variations in breast milks are least in Holder-Time pasteurized and greatest in HTST pasteurized.

It can be seen from the foregoing that cow's milk inoculated with E. coli, as far as pH variations go, keeps best when HTST pasteurized, while breast milk inoculated with E.coli keeps best when Holder-Time pasteurized.

A study of the general course of the pH values seems to indicate that E.coli has a considerably more marked effect on breast milk than on cow's milk.

V. REVIEW OF LITERATURE II

INVESTIGATIONS INTO THE BACTERICIDAL PROPERTY AND LYSOZYME OF MILK

KITASATO [122] and FOKKER [68, 69] were the first to find in fresh, raw cow's milk a bactericidal substance which had an inhibitory effect on bacterial growth. Since then several investigations have been made into the bactericidal property of both cow's milk and human milk, with very divergent results. A number of investigators have considered this substance, which they call ''inhibins'', more thermolabile than lysozyme (DOLD et al. [46], KLEINEN [124], etc.), while some speak of ''alexins'' (MORO [147] and HENNINGER [90]) and some of ''opsonins'' (WOODHEAD et al. [214]). JONES et al. [113] talk of ''lactenins'', UFFELMANN [198] and WEIGMANN et al. [202] of bacterial antagonism; HESSE [93] termed it the ''Lebensäusserung der lebenden Milch''. KLEINEN [124] and others consider milk a poor culture medium for bacteria; HEINEMANN et al. [88], BUB [29], ROSENAU et al. [166] speak of agglutinin effect or agglutination. RULLMANN et al. [169] claim that the adverse effect of milk on bacteria is attributable to its leucocyte content. JONES et al. [112] find an ''inhibitory principle'' responsible for the phenomenon. — The bactericidal effect of milk has also been considered a phenomenon of a natural and local immunity (JONES [110], BRUDNY [26], DREWES [49], GOETERS [77], GRIMMER [81], HENNINGER [90] etc.). HANSSEN [86] states that the inhibitory effect of milk bears some relationship to the oxidizing enzymes of milk; CURRAN [39, 40] asserts that the bactericidal substances of milk are connected with the circulatory system, and JONES et al. [111] assume that the inhibitory substance develops in the udders themselves. CATEL [32] claimed that breast milk contained ''secret substances''.

As some investigators have discovered in milk growth-inhibiting properties, and some properties damaging or killing bacteria, properties that have not been considered identical with lysozyme, while others have denied any bactericidal property in milk, and as the investigations into the effect of heat on these substances have also shown varying results, the author has compiled a simple table for the sake of curiosity to cover these investigations (Table I).

Lysozyme is a ferment-like substance (FLEMING [61, 62], HALLAUER [48], SURANYI [189], MEYER et al. [143]) capable of dissolving certain, particularly apathogenic bacteria FLEMING [61], [62], etc.). But it has been possible to demonstrate that it has an influence on certain pathogenic bacteria also (WOLFF [213], FLEMING [63], FLEMING et al. [65]), although ANDERSEN [5], WEISS et al. [203], THOMPSON et al. [195], KIGASAWA [121] deny the existence of any lysozyme influence whatsoever on pathogenic bacteria.

The comprehensive problem of lysozyme in general has been discussed by the following investigators: FLEMING et al. [64], ALLISON [3], NAKAMURA [151], JONES et al. [111], WOLFF [212], BORDET et al. [19], GOLDSWORTHY et al. [78], MEYER et al. [142, 143, 144], BRADFORD et al. [20], BOASSON [18], ALDERTON et al. [2], WILSON [208], WILSON et al. [209, 210], NØRREGAARD [152], BASTMAN—HEISKANEN [14].

The possibility that the ''inhibins'' may be related to or identical with lysozyme, discovered in 1922 by FLEMING [61], is under dispute. After FLEMING's [61] discovery a study was begun to ascertain whether lysozyme was present in milk too. BORDET et al. [19] found that human milk and its colostrum contain a lytic substance which persists for some time after delivery, though cow's milk and ewe's milk proved inert. The lytic power of human milk was fully active at 56°C still, 60°C weakened it slightly and a temperature of 100°C completely destroyed it. ROSENTHAL et al. [167] found lysozyme in the faeces of breast-fed but not of bottle-fed infants. It was not found in the meconium of infants during the first two days of life but was regularly present as from the fourth day in the faeces of breast-fed babies. These investigators came to the conclusion that lysozyme was present in all human milk and human colostrum samples but not in cow's milk or in the ordinary milk mixtures fed to babies. FLEMING [63] tested 12 cow's milk samples and found

Table I

Investigator	Properties inhibiting bacterial growth in fresh		Properties damaging and killing bacteria in fresh		Sensitivity to heat. Destruction temperature		All bactericidal properties in milk denied	
	human milk	cow's milk	human milk	cow's milk	in human milk	in cow's milk	human milk	cow's milk
KITASATO (122)				+		100°C		
FOKKER (68, 69)		+				70°C		
COHN & NEUMANN (38)	+							
UFFELMANN (198)								+
HONIGMANN (99)							+	
HESSE (93)				+		100°C		
WEIGMANN & ZIRN (202)								+
BASENAU (11, 13)								+
SCHOTTELIUS (177)		+						
PARK (154)		+				over 10°C		
HUNZIKER (104)		+				65°C		
MORO (146)							+	+
v. BEHRING (16)				+		100°C		
SOMMERFELD (185)								+
KOLLE (125)		+		+		60-100°C		
KONING (129)				+		100°C		
HIPPIUS (95)				+		85-100°C		
WOODHEAD & MITCHELL (214)				+				
RULLMAN & TROMMSDORFF (169)				+				
MORO (147)	+	+						
COPLANDS (37)	+	+				60-100°C		
HEINEMANN & GLENN (88) ..		+				50-100°C		
ROSENAU & McCOY (166)				+		55-80°C		

Investigator	Properties inhibiting bacterial growth in fresh: human milk	Properties inhibiting bacterial growth in fresh: cow's milk	Properties damaging and killing bacteria in fresh: human milk	Properties damaging and killing bacteria in fresh: cow's milk	Sensitivity to heat. Destruction temperature: in human milk	Sensitivity to heat. Destruction temperature: in cow's milk	All bactericidal properties in milk denied: human milk	All bactericidal properties in milk denied: cow's milk
MUCH (148)				+				
BRUDNY (26)				+				
BUB (29)		+		+				
CHAMBERS (33)		+				80-90°C		
HANSSEN (86)				+		75°C		
SCHWARTZ (179)							+	
SHERMAN & CURRAN (182)				+				
HENNINGER (90)				+				
JONES & LITTLE (111)		+				65-70°C upwards		
DREWES (49)			+	+		70-80°C		
DOLD, WIZEMANN & KLEINEN (46)		+	+		56°C	80°		
JONES (109)		+ (whey)						
JONES (110)		+				100°C		
JONES & SIMMS (112, 113)		+				80-100°C		
HAEDKE (83)	+					56-100°C		
CURRAN (39, 40)				+				
SCHLAEPPI (176)			+ (and in whey)					
KLEINEN (124)	+							
GOETERS (77)			+ (and in whey; also Holder-Time past.)			70°C upwards		
CATEL (32)			+			62-100°C		
LANNI, LANNI & BEARD (131)		+ (virus, not bact.)				fairly thermostabile		

lysozyme in all of them, although in small quantities. PRICKETT et al. [159] found that breast milk has a high lysozyme content and that the milk of the rhesus monkey, dog, cat, rat and llama revealed almost the same lysozyme concentration as human milk, the milk of the rabbit slightly less. The lysozyme content of cow's milk again was 1/100th of that of human milk, and only a trace or no lysozyme at all was found in the milk of the goat, sheep and guineapig. The faeces of infants fed with cow's milk contained no lysozyme although it was found in the faeces of sucking calves. SADAKATA [170] found that human milk has the highest lysozyme content, and next to it comes cow's milk. In aseptically taken cow's and goat's milk the effectiveness of lysozyme persists for two weeks in the refrigerator. He found that the faeces of suckled babies and of infants fed with breast milk and lactic acid milk regularly revealed lysozyme action; the faeces of infants fed with pasteurized cow's milk or goat's milk revealed no such action. DOLD et al. [46] denied the presence of lysozyme both in human milk and cow's milk. They found that sterile human milk reacts to certain bacteria in the same way as saliva and the secretion of the nose, and considered the "inhibin" effect to lie in this action. BLATT et al. [17] held the opinion that human milk contained a great deal of lysozyme, cow's milk little or none. THOMPSON [195] says that "there is no direct evidence that lysozyme is concerned in the general antibacterial activity of milk." No conclusive evidence of the existence of lysozyme in milk has been obtained by the earlier methods (THOMPSON [195], WEISS et al.[203]).

The methods applied in lysozyme investigations of milk have been very indefinite and somewhat obscure; the spectrophotometric method has not been used.

VI. PRESENT INVESTIGATIONS

MATERIAL AND METHOD

In the present investigations into the lysozyme content of milk the procedure has been as follows:

On receipt of the samples the first step was to remove the milk fat by high-speed centrifuging at 18,000 RPM (International refrigerated centrifuge model PR 1). After this the milk was passed through a Chamberland filter (Société Filtre Chamberland Système Pasteur 5 L 5); a relatively clear whey (Transmission mean 79.58 per cent) was obtained from human milk (10 samples) and a somewhat turbid whey (Transmission mean 52.03 per cent) from cow's milk (10 samples); the determinations were made with a Coleman Universal spectrophotometer model 14 turbidimetrically at a wavelength of 550 m μ.

As after preliminary experiments effected with living, 48 hours old or older bacterial strains the procedure proved unreliable, as did the investigations according to the method reported in the Preliminary Report of 1947 [181], the following method was finally adopted:

The bacteria employed for the investigation proper were Micrococcus lysodeicticus killed with 1 per cent phenol and washed according to Boasson [18]. The bacterial suspension was made in a phosphate buffer, pH = 6.2; the average Transmission value of the suspension in human milk = 2.80 per cent and in cow's milk = 2.75 per cent. Whey was placed in five tubes as follows: 5 cc into the first, 6 cc into the second, 7 cc into the third, 8 cc into the fourth and 9 cc into the fifth. 1 cc of M.lysodeikticus suspension and 0.5 per cent saline solution ad 10 cc was added to these tubes. The incubation temperature was +37° C. The optical density obtained with the Coleman apparatus was determined turbidimetrically

immediately and once every hour for four hours; the controls used consisted of whey alone, i.e. the filtered milk employed for the tests, and of the same M.lysodeikticus suspension in phosphate buffer as was employed in each of the tests. The clarification of the milk sample studied, i.e. the percentual decrease of bacteria or the lytic effect hour by hour has been calculated from the density value as a percentage of the initial value. Where simultaneous density changes were found in the control sample the density value of the samples studied was corrected accordingly before the calculation.

TEST RESULTS

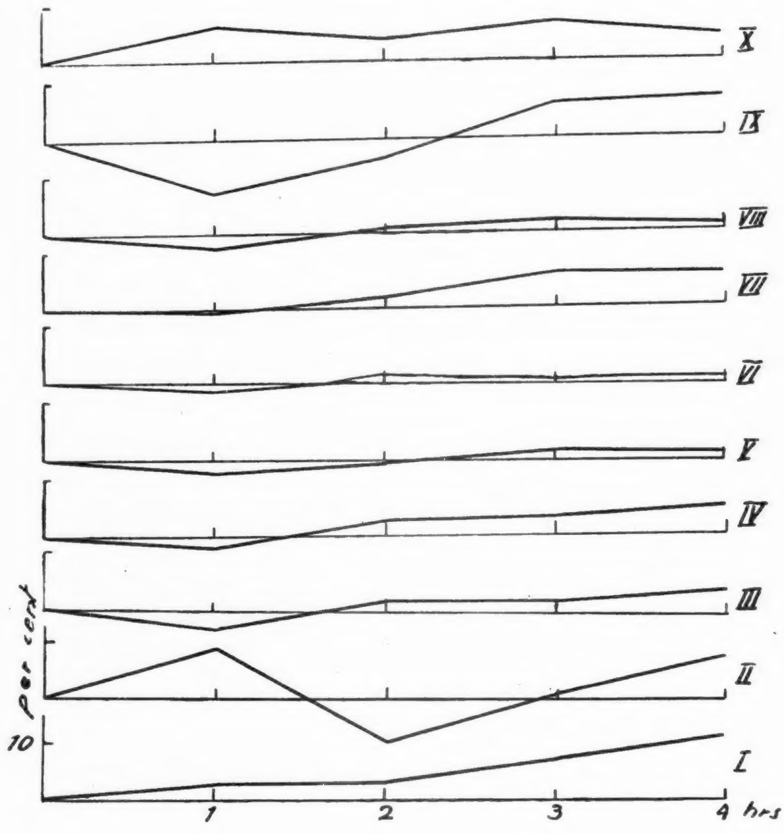
The purpose was to study whether and to what extent cow's milk and mother's milk contain lysozyme, a possible contributory factor e.g. in the keeping quality of milk. The results of the present investigation can be most distinctly seen from the first test tube series, for which reason this series only has been employed.

A. Cow's milk

Graph VII gives all 10 cow's milk samples separately and shows their effect on the M.lysodeikticus suspension. The curves are numbered in the order in which they branch off from point O. All the cows employed in the investigation are of pure Ayrshire breed, aged 3—14 years.

Review of results: In three out of ten cow's milk samples (I, II and X) a slight lytic effect on the M.lysodeikticus suspension is discernible during the first hour (minimum 2.73 — maximum 8.80 per cent). Subsequently no definite reduction of bacteria is found. In the reamaining seven samples no lytic effect is discernible during the first hour, nor is there any definite effect during the following hours although some slight clarification is subsequently noticeable.

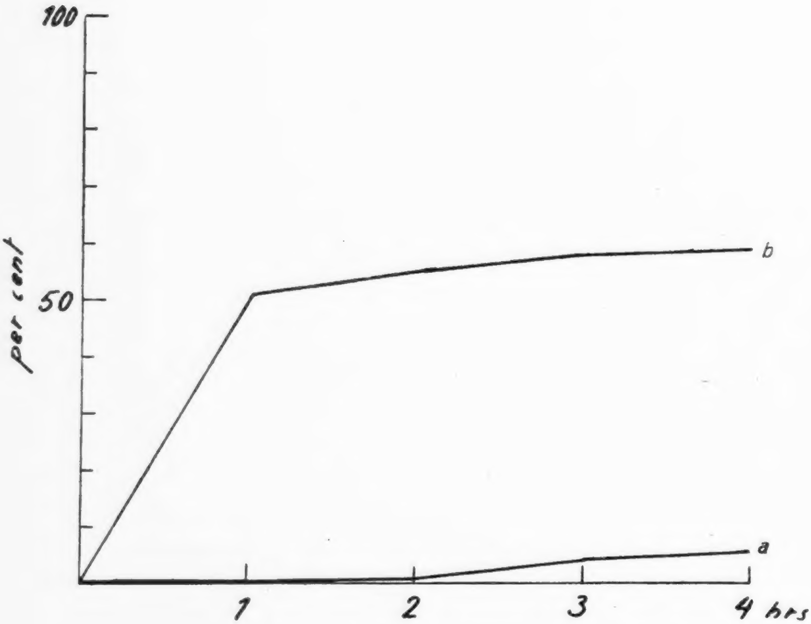
Graph VII.



Lytic effect of 10 raw cow's milk samples on killed Micrococcus lysodeikticus

Graph VIII, curve a shows the average value of the lytic effect of the cow's milk samples reported above. The curve indicates that during the first hour the cow's milk becomes clear or is capable of reducing the M.lysodeikticus bacteria contained in the suspension an average of 0.19 per cent, during the second hour 0.20 per cent, the third 3.41 per cent and the fourth hour 1.30 per cent.

Graph VIII.



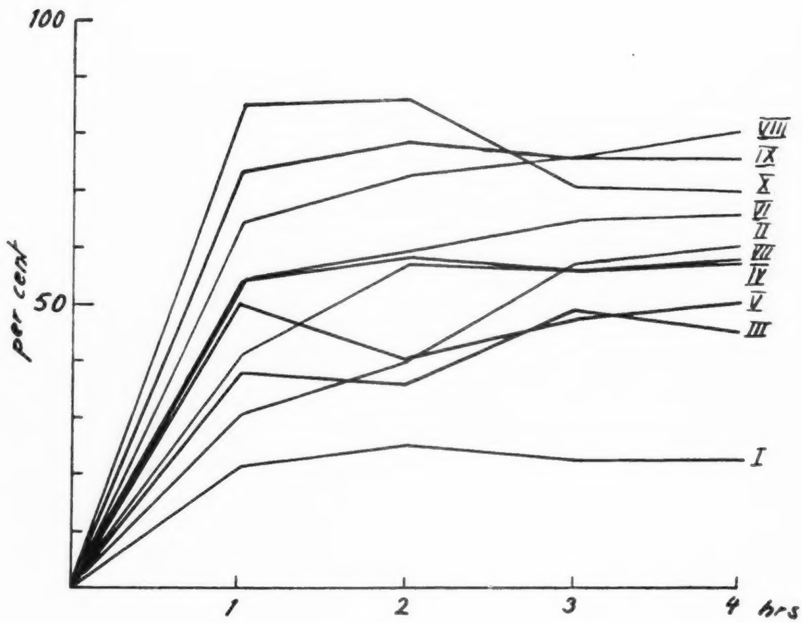
Curve a. Average lytic effect of raw cow's milk on killed Micrococcus lysodeikticus (10 samples).

Curve b. Average lytic effect of raw mother's milk on killed Micrococcus lysodeikticus (10 samples).

B. Mother's milk

Graph IX shows the tests and results with mother's milk corresponding to those in Graph VII with cow's milk. The ages of the milk donors vary between 22—41 years.

Graph IX.



Lytic effect of 10 raw mother's milk samples on killed Micrococcus lysodeikticus.

Review of results: A lytic effect is found in each breast milk sample.

Sample No.	Lytic effect	Duration of lytic effect
I	+	2 hours
II	+	Throughout the test, i.e. a minimum of 4 hours
III	+	1 hour, subsequent effect uncertain
IV	+	2 hours
V	+	1 hour
VI	+	Throughout the test, i.e. a minimum of 4 hours
VII	+	2 hours
VIII	+	Throughout the test. i.e. a minimum of 4 hours
IX	+	2 hours
X	+	2 hours

Graph VIII, curve b represents the average value of the 10 breast milk tests recorded in Graph IX. It can be seen that breast milk, on an average, is capable of dissolving 51.33 per cent of the M.lysodeikticus bacteria in the suspension during the first hour, 4.04 per cent during the second hour, 2.30 per cent during the third hour, and 1.15 per cent during the fourth hour.

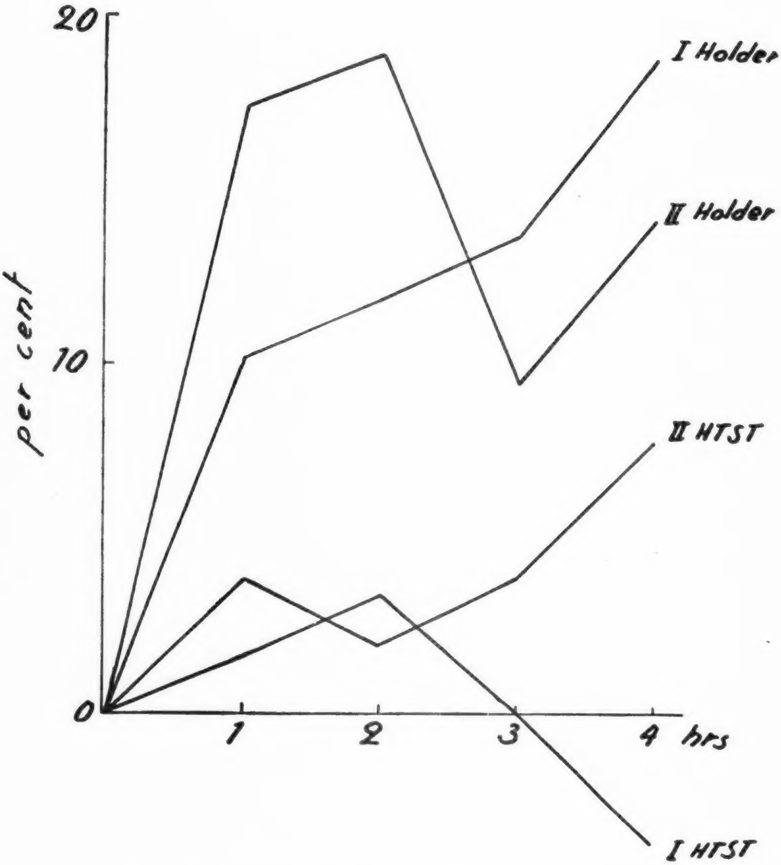
C. The effect of pasteurization on the lysozyme content of mother's milk

As the foregoing investigations have proved that mother's milk contains lysozymes in abundance but cow's milk in very small or questionable quantities and since, as mentioned earlier, Holder-time pasteurized mother's milk keeps better than HTST-pasteurized mother's milk, the author has wanted to study how these different pasteurizing methods effect the lysozyme content of mother's milk.

To clarify this point the author has determined the lysozyme content of pasteurized breast milks (2 samples each). The results are shown in Graph X.

As can be seen from Graph X, Holder-time pasteurized mother's milk clearly contains more lysozyme than HTST-pasteurized mother's milk.

Graph X.



The effect of different pasteurization methods on the lytic capacity of mother's milk (2 samples).

Summary

As regards the lysozymes in milks, cow's milk can be said to contain very small or questionable amounts of lysozyme.

The common curve for the lytic effect of 10 cow's milk samples suggests that cow's milk has a very slight lytic effect, smallest during the first hour, increasing slightly during the next hours

and then decreasing again. However, if this mean value graph is studied against the individual tests it seems that not all cow's milk samples reveal lytic activity.

The lytic effect of the breast milk of various mothers on M. lysodeikticus is somewhat individual and of varying strength; but breast milk always definitely has plenty of lysozyme.

The average lytic activity of the 10 breast milk samples is distinctly visible during the first hour and decreases subsequently hour by hour. In addition it is found that breast milk contains so much lysozyme that 5 cc of breast milk whey is capable during the first hour of dissolving an average of 51.33 per cent of the M.lysodeikticus bacteria present in 1 cc of suspension with an average Transmission value of 2.80 per cent.

Holder-Time pasteurized mother's milk contains more lysozyme than HTST pasteurized mother's milk.

VII. DISCUSSION

Cow's milk is at present pasteurized by the HTST method (either ''low pasteurization'' at 70—71° C for 15 sec. or ''high pasteurization'' at 80° C for 2 min.) since this method has generally been found better capable of killing all the bacteria usually present in cow's milk (MILLENKY et al.[145], ANDERSSON et al.[6], HOLMQVIST [97], FABIAN [58]). Several sporulating and other bacteria, especially gram-positive acid-resistant bacteria, can withstand Holder-Time pasteurization (SEIBEL et al. [180], etc.).

The author has found that HTST pasteurized cow's milk keeps better, both as regards flavour and pH changes, than Holder-Time pasteurized and raw cow's milk. On the other hand, Holder-Time pasteurized breast milk has proved to keep better, both as regards flavour and pH variations, than raw and HTST pasteurized breast milk.

An old-established fact is that breast milk generally keeps considerably better than cow's milk stored in home conditions, but no mention of this is found in the literature. By observing the changes in pH and flavour of raw cow's milk and breast milk stored for five days at room temperature and higher temperatures the author found that raw breast milk keeps better than raw cow's milk.

Kept at refrigerator temperature, the pH changes relatively little in cow's but fairly greatly in breast milk. What accounts for this difference? The reason may lie in the different composition of the bacterial flora. The fact that cow's milk and breast milk infected with Str. lactis and E. coli react in the same way to HTST pasteurization and Holder-Time pasteurization as the uninoculated milks seems to indicate that it is not the possible differences between these bacteria that accounts for the phenomenon. However, it can be assumed that alkalizing bacteria pos-

sibly dominate in raw milk at a low temperature as they generally thrive better than the acid-forming bacteria at low temperatures (BARTHEL [10] and GUBITZ [82], etc.). But why, then, does breast milk, even HTST pasteurized, become more alkaline at refrigerator temperature? It may be that not all alkali-forming enzyme systems are destroyed by this short heat treatment, and that heating for a longer time, even if at a lower temperature is required. The phenomenon is no doubt a complicated one and other enzyme factors probably also affect it.

This pH variation of relatively sterile breast milk in the refrigerator probably has some connection with the poorer buffering of breast milk. Its weak buffering capacity is evident from the fact that the dispersion of the pH values is greater than in cow's milk. The buffering capacity of cow's milk has been found to be 2—3 times higher than that of breast milk (MÜLLER [150], ARON [7], DEMUTH [42], GERSTLEY [76]), and this capacity doubtless also contributes to the pH variations (STORGÅRDS [187]). Hence breast milk is more susceptible to pH changes than cow's milk (MÜLLER [150], ARON [7], DEMUTH [42], GERSTLEY [76]).

From her present observations the author has been able to conclude that HTST pasteurization is better suited to cow's milk and Holder-Time pasteurization to breast milk. It can be thought that in addition to bacteria and buffering some other factor contributes to producing this phenomenon. That HTST pasteurized cow's milk inoculated with Str.lactis does not undergo changes in pH and flavour of the same scale as Holder-Time pasteurized and raw milk may be due to the fact that HTST pasteurization suits ''Str.lactis'' and ''E.coli milk'' better.

The present tests have shown that raw cow's milk contains very small or questionable amounts of lysozyme, and that raw breast milk has a very high lysozyme content. Is the reason for the difference in the reaction of breast milks and cow's milks under identical conditions to be found in the lysozyme?

The thermolabile lytic and bactericidal capacity of breast milk has been given several names, as mentioned above. The fact that the reduction of bacteria seen in the experiments really is due to lysozyme and not to bacteriophages, bacterial antagonism, ''inhibins'' or other bactericidal substances, emerges from the following:

Lysozyme affects both living and dead bacteria as opposed to e.g. bacteriophages which affect living bacteria only and whose properties otherwise also differ from those of lysozyme (FLEMING [61], FLEMING et al.[64]).

To eliminate the bacteriophagic effect in the experiments it was necessary to employ dead bacteria sensitive to lysozyme, i.e. M.lysodeikticus bacteria killed with 1 per cent phenol. This method, according to BOASSON [18], does not damage the bacterial structure. Hence it was possible at the same time to eliminate the possible bacterial growth during the experiments which might have led to analytical errors, and also the inhibin effect. ''Inhibins'' have a more or less growth-inhibiting effect on all living bacteria, so much so even that they may kill them (DOLD et. al. [43, 45], DOLD [44], DU DSCHENG HSING [51], IGNATIUS [105], WEIGMANN et. al. [200, 201], PESCH et al. [156]). Filtration of the milk under completely aseptic conditions eliminates the inhibin effect completely as lysozyme is filtrable (NAKAMURA [151], FLEMING [61], BORDET et al.[19], SURANYI [189]) while the ''inhibins'' are not (DOLD et al.[45]). The employment in the experiments of dead bacteria excludes the possibility of bacterial antagonism. As lysozyme is very sensitive to alkalis and acids (HALLAUER [84], FLEMING [61], NAKAMURA [151], ANDERSEN [5]) it was necessary, to avoid potential error, to employ a buffer solution with a reaction favourable to lysis and inhibiting variations in the reaction during the experiments, viz. phosphate buffer solution with a pH exceeding 6, preferably pH 6.2—6.5 (BOASSON [18], FLEMING [61], WILSON [208], SMOLELIS et al.[184], HARTSELL [87], BASTMAN—HEISKANEN [14]). As, according to FLEMING [62] and ANDERSEN [5], common salt is necessary for lysis, 0.5 per cent saline solution was employed in the experiments. According to various researchers, lysozyme is readily soluble in saline solution, indeed it is the optimal solvent for lysozyme (according to NAKAMURA [151], ½—1 per cent NaCl solution, according to FLEMING [61], 0.5 per cent and according to BORDET et al.[19], 1 pro mille—1 per cent NaCl solution).

The method of determining the lysozyme is based on lysozyme's ability to dissolve bacteria sensitive to it and hence clarify the bacterial suspension in question (FLEMING [61]). FLEMING [61] considered the clarification of bacterial suspension to be one indica-

tion of the lytic activity of lysozyme. The Coleman spectrophotometer or a similar apparatus, by which the optical density of the suspension used can be studied — the density value corresponding to a given amount of bacteria — is ideal for such an investigation (GOLDWORTHY et al.[78], THOMPSON et al.[195], BOASSON[18], SMOLELIS et al.[184], WILSON[208], BASTMAN—HEISKANEN[14]). It is also evident that optical density cannot be determined from milk as such; this is another reason why it was necessary to use whey.

In the light of the foregoing it is obvious that the effect of milk on the M.lysodeikticus suspension employed is definitely lysozyme in character. Most of the previous investigations into the lysozyme of milk have been incomplete, particularly in the method employed. This has made it impossible to judge why a number of investigators have arrived at contrary results regarding the lysozyme present in milk.

The fact that the density increases at the very outset in the cow's milk tests (samples III, IV, V, VI, VII, VIII, IX) is probably due to changes in the milk whey itself or possibly to an analytical error, and the same is true of the subsequent reductions in density also. There is hardly any question of lysozyme effect in these cases. The sample tubes were shaken before each density determination in order to make the studied solutions homogeneous.

As mentioned previously the majority of investigators consider HTST pasteurization most suitable for cow's milk, though there are some who favour Holder-Time pasteurization. Other investigators again have found no marked superiority in one method over the other.

The present investigations have shown that cow's milk keeps best HTST pasteurized and breast milk Holder-Time pasteurized; hence these milks reveal opposite reactions to different pasteurizations and differ in their keeping properties. Lysozyme can be assumed to play a part in the keeping quality of breast milk. Lysozyme is a ferment whose activity rate increases at higher temperatures ad 60°C (FLEMING[63]). The author has found that raw breast milk keeps better at higher temperatures than raw cow's milk; hence there is a possibility that the lysozyme of breast milk is activated at these higher temperatures and may perhaps

retard the souring phenomena. As the effect of lysozyme disappears or is weakened above 75° C (FLEMING [61, 62], NAKAMURA [151], SURANYI [189], BORDET et al.[19], WOLFF [212], HALLAUER [84]) and as investigations have shown that cow's milk contains no notable or very questionable amounts of lysozyme this perhaps both accounts for the fact that cow's milk keeps best HTST pasteurized and explains why the differences in pasteurization methods according to other investigators are relatively small.

As shown in Graph X considerably higher lysozyme content might be expected in Holder-Time pasteurized breast milks than is shown by these experiments. But it must be borne in mind that thermolabile enzyme systems are involved and that the sensitivity of lysozyme to heat depends on the substance containing lysozyme (FLEMING [61], [63]). The author has also observed in the present experiments that the whey of raw breast milk is considerably clearer than that of pasteurized breast milks, and in the latter the whey of Holder-Time pasteurized breast milk is somewhat clearer than that of HTST pasteurized. These changes are possibly due to denaturation, precipitation or other biochemical phenomena connected with the heating of milk. In any case lysozyme alone can hardly account for the difference in the keeping quality of cow's milk and human milk. The ultimate reasons are probably less evident. However, as more lysozyme is present in Holder-Time pasteurized human milk than in HTST pasteurized, and as Holder-Time pasteurized human milk keeps better than HTST pasteurized, it does seem as though lysozyme makes some contribution to the keeping quality of human milk.

VII. SUMMARY

1. Judged by its flavour and pH changes fresh raw uninoculated breast milk keeps better at higher temperatures (18°, 20° and 37° C) than cow's milk. On the other hand, by the same criteria, fresh raw cow's milk similarly treated keeps better than breast milk at refrigerator temperature (+ 4° C).

2. Judged by its flavour and pH changes breast milk inoculated with Streptococcus lactis keeps better at higher temperatures than cow's milk similarly inoculated. At refrigerator temperature the positions are reversed.

3. Breast milks (both raw and pasteurized) inoculated with Streptococcus lactis and Esherichia coli greatly resemble cow's milk in their pH and flavour, respectively pH variations.

4. Cow's milk keeps best HTST (High-Temperature Short-Time) pasteurized.

5. Breast milk keeps best Holder-Time pasteurized.

6. At refrigerator temperature HTST pasteurized breast milk becomes strongly alkaline, which is indicative of very great changes in this breast milk, possible due to alkalizing bacteria or various decomposition phenomena. This suggests that Holder-Time pasteurization is the preferable method of treating breast milk.

7. There is no absolute correlation between the flavour and pH of milk. No reliable conclusions as to the quality and keeping properties of milks can be drawn from the pH variations.

8. Flavour alone is not an adequate criterion for judging the quality of milk.

9. As a rule Streptococcus lactis and Escherichia coli affect breast milks more than cow's milks.

10. Fresh raw breast milk contains plenty of lysozyme.

11. Fresh raw cow's milk contains very small or questionable amounts of lysozyme.

12. The lysozyme of breast milk is most active during the first hour and weakens subsequently; the possible lytic effect of cow's milk is not a regular phenomenon.

13. Lysozyme possibly makes some contribution to the keeping quality of both raw and Holder-Time pasteurized breast milk.

IX. REFERENCES

[1] ADAMETZ, L.: Zentralbl. f. Bakt. 1890:8:109.

[2] ALDERTON, G., WARD, W. H. and. FEVOLD, H. L.: J. Biol. Chem. 1945:157:43. (Ref. Nørregaard [152]).

[3] ALLISON, V. D.: Brit. J. Exper. Path. 1924:5:165.

[4] AMERICAN ACADEMY OF PEDIATRICS, Proceedings: J. Pediat. 1943:23:112.

[5] ANDERSEN, O.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1931:70:90.

[6] ANDERSSON, A. K. and. FINCKELSTEIN, R.: J. Dairy Sci 1919: 5:347.

[7] ARON, H.: Jahrb. f. Kinderh. 1914:79:288.

[8] BAKER, J. C. and BREED, R. S.: New York State Agrl. Exper. Stat. Tech. Bull. 1920:8:3.

[9] BARTHEL, Chr.: Lait. 1921:1:62.

[10] BARTHEL, Chr.: Mikroorganismerna i lantbrukets och industrins tjänst. 1940. Sweden.

[11] BASENAU, F.: Arch. Hyg. 1895:23:44.

[12] BASENAU, F.: „ „ 1895:23:55.

[13] BASENAU, F.: „ „ 1895:23:170.

[14] BASTMAN-HEISKANEN, L.: Nord.med. 1951:46:1138.

[15] BECK, D. M., BELL, J. O., SHAW, E. W., HUEBNER, R. J.: Pub. Health Rep. 1949:63:41.

[16] von BEHRING, E.: Therap. d. Gegenw. 1904:45:1.

[17] BLATT, M. L. and KESSLER, H.: Am. J. Dis. Child. 1937:53:768.

[18] BOASSON, E. J.: J. Immunol. 1938:34:281.

[19] BORDET, M. et J.: Compt.rend. Soc. de biol. 1928:80:1252.

[20] BRADFORD, W. L. and ROBERTS, J. B.: J. Pediat. 1936:8:24.

[21] BREED, R. S.: New York State Agrl. Exper. Stat Tech. Bull. 1928:559.

[22] BREED, R. S., MURRAY, E. G. D. and HITCHENS, A. P.: B. Bergey's Manual of Determinative Bacteriology. Sixth Edition: Baltimore 1948.

[23] BROADHURST, J. and DUNCAN, J. E.: Am. J. Nursing 1933:33:453.

[24] BROCK, J.: BROCK, J., KNAUS, H., de RUDDER, B., BECKER, J., KLINKE, K.: Biologische Daten für den Kinderarzt 1939: Bd III: 180.

[25] BROOKE, M. S.: Glasgow M. J. 1947:28:378.
[26] BRUDNY, V.: Zentralbl. f. Bakt. 1909: Abt. II:22:193.
[27] BUCHBINDER, L. and FERTIG, J. W.: Nineteenth Annual Report of the New York State Association of milk Sanitarians 1945.
[28] BUCHBINDER, L. and ALFF, E. C.: J. of Milk and Food Technology 1947:10:137.
[29] BUB, M.: Zentralbl. f. Bakt. 1910: Abt. II:27:321.
[30] CATEL, W. und ZENKER, I.: Deutsche med. Wchnschr. 1940:66:959.
[31] CATEL, W.: Klin. Wchnschr. 1939:1:342.
[32] CATEL, W.: Monatschr. f. Kinderh. 1940:81:334.
[33] CHAMBERS, W. H.: J. Bact. 1920:5:527.
[34] CHAPIN, H. D.: J. A. M. A. 1917:69:886.
[35] CHRISTENSEN, J. F.: Ugeskrift før Laeger 1946:18:443.
[36] CLARK, W. M., COHEN, B. and GIBBS, H. D.: Pub. Health Rep. 1925:40:1131.
[37] COPLANDS, M.: Lancet 1907:II:1074.
[38] COHN, M. und NEUMANN, H.: Wirchows Arch. 1891:126:391.
[39] CURRAN, H. R.: J. Dairy Sci: 1931:4:276.
[40] CURRAN, H. R.: J. Infect. Dis. 1931:48:408.
[41] DAVIDSOHN, H.: Ztschr. Kinderh. 1913:9:11.
[42] DEMUTH: Ztschr. Kinderh. 1923:35:176.
[43] DOLD und WEIGMANN: Ztschr. f. Hyg. u. Infektionskr. 1934:116:158.
[44] DOLD, H.: Zentralbl. f. Bakt. 1935/36: Abt. I. Orig.: 1935.
[45] DOLD, H., LÄCHELE, W. und DU DSCHENG HSING: Ztschr. f. Hyg. u. Infektionskr. 1936:118: 369.
[46] DOLD, H., WIZEMANN, E. und KLEINEN, C.: Ztschr. f. Hyg. u. Infektionskr. 1937:119:525.
[47] DOTTERER, W. D.: Food Indust. 1940:3:74.
[48] DOTTERER, W. D.: Food Indust. 1940:12:46.
[49] DREWES, K.: Milchwirtsch. Forsch. 1927:4:403.
[50] DUDGEON, L. S. and JEWESBURY, R. C.: J. Hyg. 1924—25:23:64.
[51] DU DSCHENG HSING: Diss. Tübingen. 1935.
[52] DUNCAN, J. T. and WALKER, J.: J. Hyg. 1942:42:474.
[53] DYNSKI—KLEIN, M.: Brit. M. J. 1946:II:258.
[54] EMERSON, P. W. and PLATT, W.: J. Pediat. 1933:2:472.
[55] ESCHERICH, Th.: Fortschr. d. Med. 1885: Bd 3:8:231.
[56] ESCHERICH, Th.: Fortschr. d. Med. 1895:8:2.
[57] ESTEVES, C.: Pediatrics section VII of Excerpta Medica 1949: Vol. III:2.
[58] FABIAN, F. W.: J. Milktech. 1942:5:237.
[59] FEER, E.: Lehrbuch der Kinderheilkunde 1948:16:39.
[60] FISCHER, H.: Mitt. a.d. Geb. d. Lebensmittelunt. u. Hyg. (Schwz) 1947:38:48.
[61] FLEMING, A.: Proc. Roy. Soc. London, s. B. 1922:93:306.

62 FLEMING, A.: Lancet 1929:I:217.
63 FLEMING, A.: Proc. Roy. Soc. Med. 1932:26:71.
64 FLEMING, A. and ALLISON, V. D.: Brit. J. Exper. Path. 1922:3:252.
65 FLEMING A. and ALLISON, V. D.: Lancet 1924:206:1303.
66 FLEMING, A. and ALLISON, V. D.: Brit. J. Exper. Path. 1927:8:214.
67 FLÜGGE, C.: Arch. Hyg. 1894:17:272.
68 FOKKER, A. P.: Fortschr. d. Med. 1890:1:7.
69 FOKKER, A. P.: Ztschr. f. Hyg. u. Infektionskr. 1890:9:41.
70 FREI, W. und LIENHARD, H.: Biochem. Ztschr. 1926:178:1.
71 GAIRDNER, P.: Arch. Dis. Childhood 1945:20:22.
72 GARLAND, J.: Boston M. & Surg. J. 1923:189:563.
73 GARLAND, J.: Ibid. 1923:189:460.
74 GARLAND, J.: Ibid. 1923:189:513.
75 GARLAND, J.: Ibid. 1923:189:975.
76 GERSTLEY, J. R.: Am. J. Dis. Child. 1933:45:538.
77 GOETERS, W.: Ztschr. f. Kinderh. 1940:61:184.
78 GOLDSWORTHY, N. E. and FLOREY, H.: Brit. J. Exper. Path. 1930:11:192.
79 GOTSCHLICH, E.: KOLLE, W., KRAUS, R. und UHLENHUTH, P.: Handbuch der pathogenen Mikro-organismen 1929: Bd. I:1:332.
80 GOULD, I. A. and SOMMER, H. H.: Tech. Bull. 1939:164:2.
81 GRIMMER, W.: Chemie und Physiologie der Milch, Berlin 1910.
82 GUBITZ, H.: Milchwirtsch. Forsch. 1928: Orig. Bd. 5:407.
83 HAEDKE, K.: Diss. Tübingen 1929.
84 HALLAUER, C.: Zentralbl. f. Bakt. Orig. 1929:114:519.
85 HALLMAN, N.: Duodecim 1951:2:146.
86 HANSSEN, F. S.: Brit. J. Exper. Path. 1924:5:271.
87 HARTSELL (ref. SMOLELIS & HARTSELL [184]).
88 HEINEMANN, P. G. and GLENN, T. H.: J. Infect. Dis. 1908:5:534.
89 HELSKE, M.: Personal communication.
90 HENNINGER, E.: Arch. Hyg. 1926:97:183.
91 HERWICK, M. K.: Am. J. Nursing. 1933:33:454.
92 HESSE, W.: Ztschr. f. Hyg. u. Infektionskr. 1893:13:42.
93 HESSE, W.: Ztschr. f. Hyg. u. Infektionskr. 1894:17:238.
94 HILEMAN, J. L., MOSS, C. and STEAD, B.: J. Dairy Sci 1941:24:799.
95 HIPPIUS, A.: Jahrb. f. Kinderh. 1905:61:365.
96 HOLMES, A. D.: Am. J. Dis. Child. 1949:78:899.
97 HOLMQVIST, S. A.: Ann.rept. 1932:20:79. (Ref. MILLENKY & BRUECKNER [145]).
98 HOLWERDA, B. K.: Biochem. Ztschr. 1922:128:465.
99 HONIGMANN, F.: Ztschr. f. Hyg. u. Infektionskr. 1893:14:205.
100 HONIGMANN, M. und TROMMSDORFF, R.: Ztschr. f. Hyg. u. Infektionskr. 1893:14:205.
101 HUCKER, G. J.: New York State Agrl. Exper. Stat. Tech. Bull. 1928:134.

102 HUEBNER, R. J., JELLISON, W. L., BECK, M. D., PARKER, R. P., SHEPARD, C. C.: Pub. Health Rep. 1948:63:214.

103 HUEBNER, R. J., JELLISON, W. L., BECK, M. D., and WILCOX, F. P.: Pub. Health Rep. 1949:64:499.

104 HUNZIKER, O.: Ref. Zentralbl. f. Bakt. 1902:Abt.II:9:874.

105 IGNATIUS, A.: Ztschr. f. Hyg. u. Infektionskr. 1936:118:445.

106 JELLISON, W. L., ORMSBEE, R., BECK, M. D., HUEBNER, R. J., PARKER, R. R., BELL, E. J.: Pub. Health Rep. 1948:63:1611.

107 JIMISON, R. L.: Mil. Surgeon 1947:6:507.

108 JOHANNSEN, A.: Hyg. Revy 1947:177.

109 JONES, F. S.: J. Exper.Med. 1928:47:877.

110 JONES, F. S.: Proc. A.A.M.M.C.XXIII Ann.Conference 1929:202.

111 JONES, F. S. and LITTLE, R. B.: J.Exper.Med. 1927:45:319.

112 JONES, F. S. and SIMMS, H. S.: J.Exper.Med. 1929:50:279.

113 JONES, F. S. and SIMMS, H. S.: J.Exper.Med. 1930:51:327.

114 JOSEPHSON, D. V. and DOAN, F. J.: Milk Dealer 1939: nov.: 35—36 and 54.

115 KAY, H. D.: Science Progress 1949:148:609.

116 KAYSER, M.-E.: Arch.Gyn. 1938:166:417.

117 KAYSER, M.-E.: Arch.Gyn. 1938:166:472.

118 KAYSER, M.-E.: Frauenmilchsammelstellen, Jena 1940.

119 KEENEY, D. G., BAUR, L. S. and GARRETT, O. F.: J. Pediat. 1949: 34:745.

120 KELLEY and OSBORNE (ref. GARLAND [74]).

121 KIGASAWA, T.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1927/1928:54:155.

122 KITASATO, S.: Ztschr. f. Hyg. u. Infektionskr. 1889:5:491.

123 KITCHEN, J. M. W.: M.Rec. 1919:95:725.

124 KLEINEN, C.: Diss. Tübingen 1938.

125 KOLLE, W.: Klin. Jahrb. 1905:13:317.

126 KOLLE, W.: KOLLE, W., KRAUS, R. und. UHLENHUTH, P.: Handbuch der pathogenen Mikro-organismen 1929:Bd I:1:357.

127 KON, S. K.: Brit. M. Bull. 1947:5.

128 KON, S. K. and MAWSON, E. H.: Proc.Roy.Soc.Med. 1947:40:900.

129 KONING, C. I.: Ref. Zentralbl. f. Bakt. 1905:Abt..II:14:424.

130 KÄSTLI, P.: Mitt. a.d. Geb. d. Lebenmittelunt. u. Hyg. (Schwz) 1949:XXXIX:46.

131 LANNI, F., LANNI, Y. T. and BEARD, J. W.: Proc.Soc. Exper. Biol. & Med. 1949:72:1:227.

132 LAWS, C. H. and SKELLEY, E. G.: Am.J.Nursing. 1938:38:859.

133 LAWS, C. H. and SKELLEY, E. G.: Am.J.Nursing. 1938:38:1003.

134 LAWS, C. and SKELLEY, E. G.: Am.J.Nursing. 1939:39:381.

135 LEISTI, L.: Duodecim 1949:717.

136 LEISTI, L.: Duodecim 1950:679.

137 MACKINTOSH, J. M.: Brit. M. Bull. 1947:5.

[138] MACPHERSON, C. H. and TALBOT, F. B.: J. Pediat. 1939:15:461.

[139] MASON, F. W.: J. Roy. San. Inst. 1949:LXIX:569.

[140] McCULLOCH, E. C.: Disinfection and Sterilization. Philadelphia 1945.

[141] MELLANDER, O.: Upsala Läkareförenings förhandlingar 1946—47: 52:108.

[142] MEYER, K., HAHNEL, E. and. STEINBERG, A.: J.Biol.Chem. 1946: 163:733.

[143] MEYER, K., THOMPSON, R., PALMER, J. W. and KHORAZO, D.: J.Biol.Chem. 1936:113:303.

[144] MEYER, K., THOMPSON, R., PALMER, J. W., KHORAZO, D.: Science 1934:79:61.

[145] MILLENKY, A. and BRUECKNER, H. J.: Cornell Univ. Agrl. Exper. Stat. 1941:754.

[146] MORO, E.: Jahrb. f. Kinderh. 1902:55:396.

[147] MORO, E.: Ztschr. f. exper. Path. u. Therap. 1907:4:470.

[148] MUCH, H.: Münch.Med.Wchnschr. 1908:8:384.

[149] MÜLLER, F.: Ztschr. f. Kinderh. 1923:35:285.

[150] MÜLLER, W.: Jahrb. f. Kinderh. 1892:34:439.

[151] NAKAMURA, O.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1923/1924:38:425.

[152] NØRREGAARD, S.: Nord.med. 1950:44:1923.

[153] OBERMEIER, W.: Diss. Hanau 1919.

[154] PARK, W. H.: J.Hyg. 1901:1:391.

[155] PASTEUR, L.: (Ref. McCULLOCH [140]).

[156] PESCH, K. L. und DAMM, R.: Ztschr. f. Hyg. u. Infektionskr. 1936:118:1.

[157] PESCH, K. L. und SIMMERT, U.: Milchwirtsch. Forsch. 1929:8: 551.

[158] POETSCHKE, G.: Monatschr. f. Kinderh. 1950:98:177.

[159] PRICKETT, P. S., MILLER, N. J. and McDONALD, F. G.: J. Bact. 1933:25:61.

[160] PRUDDEN, T. M.: M.Rec. 1890:37:85.

[161] PUBLIC HEALTH SERVICE MILK ORDINANCE AND CODE 1935: Pub. Health Bull.1935:220:76. U.S. Treasury Department Public Health Service, Washington, D.C.

[161 a] RANTASALO, V.: Personal communication.

[161 b] RANTASALO, V.: Acta Paediat. 1949:LXXVII:209.

[162] REPORT OF THE SPECIAL MILK BOARD OF THE MASSACHUSETTS STATE DEPARTMENT OF HEALTH. Boston 1916.

[163] RITTER, W.: Mitt. a.d. Geb. d. Lebenmittelunt. u. Hyg. (Schwz) 1949:40:43.

[164] ROADHOUSE, C. L. and. KOESTLER, G. A.: J. Dairy Sci 1929:XII: 421.

[165] ROADHOUSE, C. L.: Milk Dealer 1939:nov.:44.

166 ROSENAU, M. J. and McCOY, G. W.: J. M.Res.1908:18:165.
167 ROSENTHAL, L. and LIEBERMAN, H.: J.Infect.Dis.1931:48:226.
168 ROWLANDS, A.: Brit.M.Bull. 1947:5:1115.
169 RULLMANN, W. und TROMSDORFF, R.: Arch.Hyg.1906:59:224.
170 SADAKATA, K.: Am.J.Dis. Child. 1933:45:1360.
171 SALMI, T.: Acta Paediat. 1939:XXIV:111.
172 SAUER, W.: Deutsche med. Wchnschr. 1939:65:1691.
173 SAVAGE, W.: Brit.J.Soc.Med. 1949:3:45.
174 SCHERER, P.: Ztschr. f. Hyg. 1951:132:217.
175 SCHEUER, L. A. and DUNCAN, J. E.: Am J.Dis.Child. 1936:51:249.
176 SCHLAEPPI (ref. BLATT & KESSLER [17]).
177 SCHOTTELIUS, M.: Zentralbl. f. Bakt. 1896:20:897.
178 SCHULTZ, E. W., MARX, A. and BEAVER, H. J.: J. Dairy Sci 1922: 5:385.
179 SCHWARZ, G.: Deutsche med. Wchnschr. 1924:50:754.
180 SEIBEL und HENNEBERG: Milchwirtsch. Forsch. 1926:3:309.
181 SELESTE, E.: Ann.Med.Int.Fenn. 1947:36:630.
182 SHERMAN, J. M. and CURRAN, H. R.: Proc.Soc.Exper.Biol. & Med. 1924/25:22:15.
183 SMITH, C. A.: J. Pediat. 1942:20:616. (Ref. WRIGHT [204]).
184 SMOLELIS, A. N. and HARTSELL, S. E.: J. Bact. 1949:58:731.
185 SOMMERFELD, P.: Zentralbl. f. Bakt. 1904:Abt.I:37:716.
186 DE SORIANO, A. M.: Rev.Asoc.argent.dietol. 1946:IV:13:63.
187 STORGÅRDS, T.: Personal communication.
188 STRAUS, N.: Ref. Boston Med.a.surg. J. 1923:188:748.
189 SURANYI, L.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1927: 49:166.
190 v. SYDOW, G.: Svenska Läkartidn. 1948:20.
191 SZILI (Ref. OBERMEIER [153]).
192 TALBOT (Ref. WHRIGHT [204]).
193 TITUS, E. W.: M.Rec. 1914:86:58.
194 THOMÉ, K. E.: Bakteriologiska synpunkter på reduktasprovet, Meddelande N:o 7 från Statens Mejeriförsök, Malmö 1941.
195 THOMPSSON, R. and KHORAZO, D.: Proc.Soc.Exper. Biol. & Med. 1935—36:33:299.
196 THOMPSSON, R.: Arch.Path. 1940:30:1096.
197 THÖNI, J.: Zentralbl. f. Bakt. 1914:Abt.I.Orig.Bd.:74:11.
198 UFFELMANN, J.: Berl. klin. Wchnschr. 1892:29:1209.
199 WEDEMAN: Zentralbl. f. Bakt. 1926: Abt. I. Orig. Bd. 97:50.
200 WEIGMANN, F. und KOEHN, A.: Ztschr. f. Hyg. u. Infektionskr. 1936:118:507.
201 WEIGMANN, F. und KOEHN, A.: Ztschr. f. Hyg. u. Infektionskr. 1936:118:516.
202 WEIGMANN, H. und ZIRN, G.: Zentralbl. f. Bakt. 1894:15:286.
203 WEISS, J. E. and RETTGER, L. F.: J.Bact. 1934:28:515.

[204] WHRIGHT, J.: Month.Bull.Min.Health 1947:6:74.
[205] WHRIGHT, J.: Lancet 1947:2:121.
[206] WHRIGHT, J. and EDVARDS, E.: Lancet 1947:2:233.
[207] WILSON, G. S.: Brit.M.J. 1943:1:261.
[208] WILSON, A. T.: J. Pediat. 1950:36:39.
[209] WILSON, A. T. and HADLEY, W. P.: J. Pediat. 1950:36:45.
[210] WILSON, A. T. and HADLEY, W. P.: J. Pediat. 1950:36:199.
[211] WOLF, K.: Ztschr. Fleisch- u. Milchhyg. 1928:38:169.
[212] WOLFF, L. K.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1927:50:88.
[213] WOLFF, L. K.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 1927/1928:54:188.
[214] WOODHEAD, G. S. and MITCHELL, W. A.: J.Path. & Bact. 1906: II:408.
[215] YLPPÖ, A.: v. PFAUNDLER, M. und SCHLOSSMANN, A.: Handbuch der Kinderheilkunde 1931: Aufl. IV. Bd. I:575.
[216] YLPPÖ, A., HALLMAN, N., DONNER, M., LOUHIVUORI, K., NEVANLINNA, E., YLIRUOKANEN, A.: Ann.Med.Int.Fenn.: 1950:39:149.

INDEX

Vol. 31.

Suppl. 1

Suppl. 2

Suppl. 3

Hirsjärvi, Eva: Erythrocytosis-Promoting Activity in Stagnant Blood and in Blood Subjected to Low Athmospheric Pressure *in vitro*

Suppl. 4

Järvinen, Pentti A.: Über die Pharmakodynamischen und Klinischen Wirkungen des Finnischen Mutterkorns

Suppl. 5

Widholm, Olof: Studies on *Escherichia coli* Hemolysins and Antihemolysins

Suppl. 6

Kalliomäki, Lennart: Observations Based on Syphilis-Serologically Studied Material

Suppl. 7

Paasonen, M. K.: Effect of Some Antihistaminics on Adrenaline Responses in Animal Tests

Suppl. 8

Seleste, Ester: The Keeping Quality of Cow's Milk and Mother's Milk